CONGENITAL MUSCULAR DYSTROPHY

Clinical and morphological studies

Q.H. LEIJTEN

CONGENITAL MUSCULAR DYSTROPHY

Clinical and morphological studies

CONGENITAL MUSCULAR DYSTROPHY

Clinical and morphological studies

Een wetenschappelijke proeve op het gebied van de
Medische Wetenschappen

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Katholieke Universiteit Nijmegen,
volgens besluit van het College van Decanen
in het openbaar te verdedigen op vrijdag 19 januari 1996,
des namiddags te 1.30 uur precies

door

Quinten Harm Leijten

geboren op 11 november 1954 te Tilburg

Promotor : Prof.Dr. F.J.M. Gabreëls
Co-promotores : Dr. W.O. Renier
Dr. H.J. ter Laak

This study was performed at the Department of Child Neurology, Institutes of Neurology and Pediatrics, University of Nijmegen. The study was part of the research programme 'Disorders of the Neuromuscular System' of the University of Nijmegen.

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Leijten, Quinten Harm

Congenital muscular dystrophy: clinical and morphological studies / Quinten Harm Leijten. - [S.l. : s.n.]. - Ill.
Proefschrift Nijmegen. - Met samenvatting in het Nederlands.
ISBN 90-9009035-5
Trefw.: congenitale spierdystrofie.

"I esteem it the office of the physician not only
to restore health but to mitigate pain and dolors."

SIR FRANCIS BACON

aan Lizette, Eline, Derek en Sophie

Drukwerk SSN, Nijmegen

Publication of these thesis was financially supported by

ASTA Medica, Bayer B V , Boehringer Ingelheim, Byk, Christiaens B V , Ciba-Geigy, Duphar, Eli-Lilly, Ethifarma, A Sigma Tau Company, Glaxo B V , Hoechst Roussel, Katwijk farma, MSD, Novo Nordisk Farma, Roche Nederland B V , Roche Nicholas B V , Sandoz Pharma B V , Sanofi Winthrop v o f , UCB Pharma, Upjohn-Nederland, Yamanouchi Pharma B V

Contents

Abbreviations

ABC	avidin-biotin complex
BAEP	brainstem auditory evoked potential
CK	creatine kinase
CMD	congenital muscular dystrophy
CNS	central nervous system
COD-MD	cerebro-ocular dysplasia-muscular dystrophy
CSF	cerebrospinal fluid
CT	computed tomography
CV	conduction velocity
DAG	dystrophin-associated glycoprotein
DAP	dystrophin-associated protein
ECG	electrocardiography
EEG	electroencephalography
EMG	electromyography
ENMC	European Neuromuscular Centre
ERG	electroretinography
F-CMD	Fukuyama type of congenital muscular dystrophy
F-MEB-D	Finnish type of muscle, eye and brain disease
GFAP	glial fibrillar acid protein
MBP	myelin basic protein
MEB-D	muscle, eye and brain disease
MNCV	motor nerve conduction velocity
MRI	magnetic resonance imaging
MRS	magnetic resonance spectrometry
NCV	nerve conduction velocity
nF-CMD	non-Fukuyama type congenital muscular dystrophy
NSE	neuron-specific enolase
O-CMD	occidental-type cerebromuscular dystrophy
OFC	occipitofrontal circumference
Pure-CMD	'pure' congenital muscular dystrophy
SCARMD	severe childhood autosomal recessive muscular dystrophy
SD	standard deviation
S(S)EP	somatosensory evoked potential
VEP	visual evoked potential
WWS	Walker-Warburg syndrome

Chapter I

OUTLINE OF INVESTIGATION

This study aimed to present the results of our own investigations on patients whose clinical and pathological features met the criteria for the diagnosis of congenital muscular dystrophy (CMD) and to compare these findings to those reported in the literature (Chapter II).

Materials

Clinical criteria that suggested the diagnosis of CMD were: (1) generalized congenital hypotonia ('floppy infant'), (2) congenital muscle weakness and (3) contractures ('arthrogryposis'). The diagnosis suspected on clinical grounds was confirmed by muscle biopsy, which showed dystrophic characteristics.

Over the past 16 years, 3500 muscle biopsies have been examined at the neuromorphological department. CMD was found in 34 of them. Eighteen cases, including four with white matter hypodensity (type IB), had classical or 'pure' CMD without severe impairment of their intellectual development (type I). Structural brain abnormalities and mental retardation (Fukuyama type CMD, F-CMD, type II) were present in eight cases. Two exceptional cases were classified as F-CMD-like cases. Six cases had structural brain and eye abnormalities (the Walker-Warburg syndrome (WWS), type IV). Autopsy of the brain was performed on four cases with WWS, on two cases with F-CMD and on one of the patients with the F-CMD-like condition. We also added some cases with particular forms of muscle dystrophy to the series.

Methods

The study was primarily a clinico-morphological study on CMD. We compared the clinical and morphological aspects recorded in our patients to those reported in the literature. Some (semi)-quantitative examinations of morphological changes were performed on muscle biopsies. The results have been published in various articles (Chapters III and IV). In the final chapter (Chapter V), we discuss our results in the light of the most recent CMD classification from 1995.

Chapter II

CONGENITAL MUSCULAR DYSTROPHY:

A REVIEW OF THE LITERATURE

Q.H. Leyten, F.J.M. Gabreëls, W.O. Renier, H.J. ter Laak

Submitted for publication

Abstract

The name congenital muscular dystrophy (CMD) has been given for a condition in which there is already at birth marked hypotonia, generalized muscle weakness and frequently multiple contractures. CMD has recently been classified into four categories: classical or 'pure' CMD without severe impairment of intellectual development (CMD I), the Fukuyama type CMD with muscle and structural brain abnormalities (CMD II), CMD with muscle, eye and brain abnormalities: the Finnish type CMD (CMD III) and the more severe Walker-Warburg syndrome (CMD IV). Data of the literature concerning those different CMD types have been reviewed and are presented with emphasis on signs and symptoms, clinical course, laboratory, neurophysiological, radiological, morphological and genetic characteristics.

Historical aspects and recent classification

Since Oppenheim [1] first described generalized muscle weakness in early childhood in 1900, calling it 'amyotonia congenita', many papers have appeared on a heterogeneity of disorders that not only include primary muscle atrophies, but also secondary and neurogenic muscle atrophies [2,3]

In 1903, Batten [4] described a congenital muscle disorder in a boy that was characterized by proximal weakness, torticollis, flexion contractures at the knees, hips and elbows, scoliosis and kyphosis The boy died at the age of 7 years Neuropathological examination did not reveal any abnormalities in the central nervous system, but primary alterations were found in the muscles that corresponded with muscular dystrophy

The term congenital muscular dystrophy (CMD) was first used by Howard [5] in 1908 He described a neonate with dystrophic changes in the muscles and introduced the term 'dystrophia muscularis congenita' Afterwards, several other cases of CMD were recorded [6-10] After the reports by Ullrich [9,10] in 1930, only a few cases have appeared in the literature, limited to Germany [11-13] and Japan [14-21] In 1954, Walton and Natrass [22] recognized CMD as a clinically and genetically distinctive entity with an autosomal recessive mode of transmission

In the late fifties and early sixties, some CMD cases were described as myopathic cases of 'arthrogryposis multiplex' [23-29]

In 1960, Fukuyama et al [30] described a distinctive type of CMD with cerebral malformations The incidence of this disorder in Japan is about half as frequent as Duchenne muscular dystrophy [31], but is fairly rare in countries outside Japan [32-41]

Santavuori et al [42] and Raitta et al [43] introduced the term 'muscle, eye and brain disease' (MEB-D) in 1977 and 1978, respectively The term was used to represent another distinct type of congenital muscular dystrophy associated with central nervous system and eye anomalies Santavuori et al were not the first to describe an eye-brain syndrome In 1942, Walker [44] described a hydrocephalic child with lissencephaly and maldevelopment of both eyes, however, the muscles were not analyzed In 1978, Warburg [45,46] described cases with retinal nonattachment associated with hydrocephalus in her review of the literature Based on Warburg's clinical description of the disorder, Pagon et al [47] introduced the term HARD +/– E syndrome as a mnemonic for hydrocephalus, agyria and retinal dysplasia with or without encephalocele, but later [48,49] withdrew this mnemonic and suggested the name Warburg syndrome or,

according to Dobyns et al. [50], Walker-Warburg syndrome. When in 1984, Towfighi et al. [51] found typical dystrophic muscular changes in cases with the Walker-Warburg syndrome, Dobyns et al. [52,53] suggested that there was only a gradual distinction between the Walker-Warburg syndrome and MEB-D as described by Santavuori et al. [42] and Raitta et al. [43].

Since 1979, there have been reports on patients with CMD with normal or subnormal cognitive functioning whose CT scans and/or MRI images indicate marked hypodensity of the white matter [37,40,54-67]. Castro-Gago and Peña-Guitián [57] and Topaloğlu et al. [61,62] suggested that this type should be considered as an intermediate form between the 'pure' CMD and the Fukuyama type of CMD in 1988 and in 1990 and 1991, respectively. They proposed the term 'occidental type cerebromuscular dystrophy'. The recent discovery of merosin (merosin M-chain or laminin-α_2) deficiency, an extracellular matrix protein, in patients with 'pure' CMD and marked hypodensity of the white matter, has enabled the identification of two subgroups of 'pure' CMD patients according to the presence or the absence of merosin [68,69].

In order to delineate the different nosological entities, international workshops were organized by Dubowitz in 1993 and 1994 [67,70], which resulted in a general proposal for the classification of CMD (Table 1).

Table 1. The 1995 CMD classification according to the literature and two ENMC sponsored workshops [67,70]

CMD I	Classical or 'pure' CMD without severe impairment of intellectual development.
	A. Without white matter hypodensity and with normal normal merosin (merosin M-chain or laminin-α_2) expression (McKusick, no. 23667),
	B. With white matter hypodensity and with deficient merosin (merosin M-chain or lamina-α_2) status.
CMD II	CMD with mental impairment due to structural brain abnormality: the Fukuyama type (McKusick, no. 253800)
CMD III	CMD with eye and brain abnormalities: the Finnish type (McKusick, no. 253280)
CMD IV	CMD with eye and brain abnormalities: the Walker-Warburg syndrome (McKusick, no. 236670).

Common clinical characteristics and muscle pathology

Clinical characteristics (Table 2)

The name congenital muscular dystrophy (CMD) is given to an autosomal recessive condition in which marked hypotonia is already present at birth or has an onset during the first year of life, frequently multiple contractures (arthrogryposis) and more or less generalized muscular weakness. The muscles of the pelvic and shoulder girdles and of the proximal limbs are predominantly affected, but in many patients involvement of the neck and facial muscles is found [71,72]. Clinical course is non-progressive or slowly progressive [71,72].

Muscle pathology (Table 2)

The myopathological changes in CMD are variable. They include variation in fibre size, an increased number of fibres with internal nuclei and a marked increase in interstitial connective tissue with or without an increase in interstitial adipose tissue (Fig. 1A). No marked fibre necrosis and regenerative activity are seen in comparison with other muscular dystrophies, such as Duchenne muscular dystrophy and limb-girdle dystrophy, and evidence may even be lacking in the biopsy. However, sometimes more pronounced dystrophic patterns are found with evident necrosis and regeneration. Both fibre types are affected, but not always equally. The pathological changes can vary in severity between muscles and even between fascicles [30,31,40,42,52,66,67,70-72].

CMD: characteristics of different types of congenital muscular dystrophy

In the following paragraphs, signs, symptoms, clinical course, laboratory, neurophysiological, radiological, morphological and genetic characteristics of the different types of CMD are outlined.

Table 2. Diagnostic criteria for classic or 'pure' CMD

Inclusion criteria	Exclusion criteria
Clinical features	Clinical features
– onset at birth or during the first year of life,	– onset during childhood,
– hypotonia and generalized muscular weakness,	– clinical course rapidly progressive,
– multiple joint contractures evident in the first year of life,	– muscular hypertrophy,
– clinical course non-progressive or slowly progressive,	– ptosis and/or ophthalmoplegia,
– normal or subnormal mental development	– severe impairment of intellectual development (IQ <50),
	– structural ocular abnormalities
Muscle biopsy	Laboratory findings
– dystrophic pattern	– EMG with 'neuropathic' pattern,
– marked increase in interstitial connective tissue with or without increase in interstitial adipose tissue,	– muscle biopsy normal, with neuropathic abnormalities or with structural abnormalities specific of other myopathies,
– no marked fibre necrosis and regenerative activity,	– dystrophin absent or abnormal,
– sometimes more pronounced by dystrophic pattern, with evident necrosis and regeneration , dystrophin (both by immunocytochemical and Western-blot techniques) must be present and normal	– major CT or MRI abnormalities of CNS different from white matter hypodensity (major malformations, developmental and/or migration defects)

CMD I. Classic or 'pure' form of congenital muscular dystrophy without severe impairment of intellectual development

CMD IA. Classic or 'pure' form of congenital muscular dystrophy without severe impairment of intellectual development, without white matter hypodensity and with normal merosin (merosin M-chain or laminin-α_2) expression

Signs, symptoms and clinical course [40,67,70-72] (Table 2)

In many patients, decreased antenatal movement is noted. Postnatal asphyxia can occur. Signs and symptoms are present at birth or become apparent during the first year of life. Most patients are hypotonic at birth with generalized weakness (Fig. 2A). In most patients, the proximal muscles are more affected than the distal ones and initially the arms are more impaired than the legs. Facial, neck and chest musculature is variably involved, while the sucking and swallowing musculature and extraocular muscles are spared. Ophthalmologic examination is normal. The tendon reflexes are usually depressed or absent. In all patients, motor development is significantly delayed. However, the clinical course is not usually very progressive and differs from case to case. In some patients, contractures occur at birth and in others at a later age. In some patients, kyphoscoliosis of the thoracolumbar spine is present [40]. Also congenital subluxation or dislocation of the hips and clubfeet are common features. Mental development is usually normal (or subnormal). The degree of impairment and the delay in passing motor milestones vary from case to case. Most patients are able to sit up and one in three is able to stand at 5 years of age [73,74]. Most patients do not reach adolescence and die from progressive muscle weakness and respiratory failure. In general, patients who lack the ability to handle their pulmonary secretions are prone to developing frequent pulmonary infections and many of them die as a result. The prevalence of CMD abroad is estimated at 1 : 60,000 at birth and at 1 : 100,000 in the population [75].

Laboratory findings

Serum creatine kinase (CK) values are usually normal or moderately increased. Increased values usually tend to return to normal with increasing age, but not always [71].

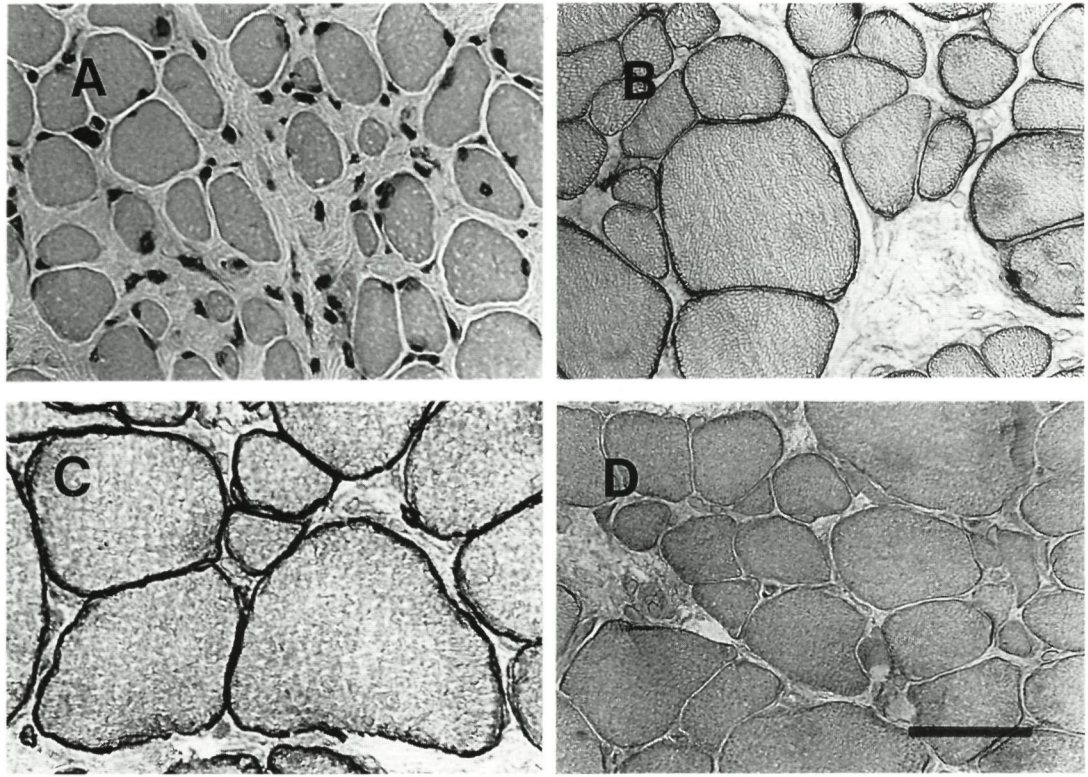

Fig. 1. Muscle biopsies of 4 patients stained for HE (A), dystrophin (B) and merosin (C and D). In 'pure' CMD, dystrophin expression is normal (B), but merosin expression may be normal (C) or deficient (D). Patient ages: 3 (A, B and C) and 1 year (D). Biopsy from quadriceps muscel (A, C and D) or rectus abdominis muscle (B). Bar = 50 μm.

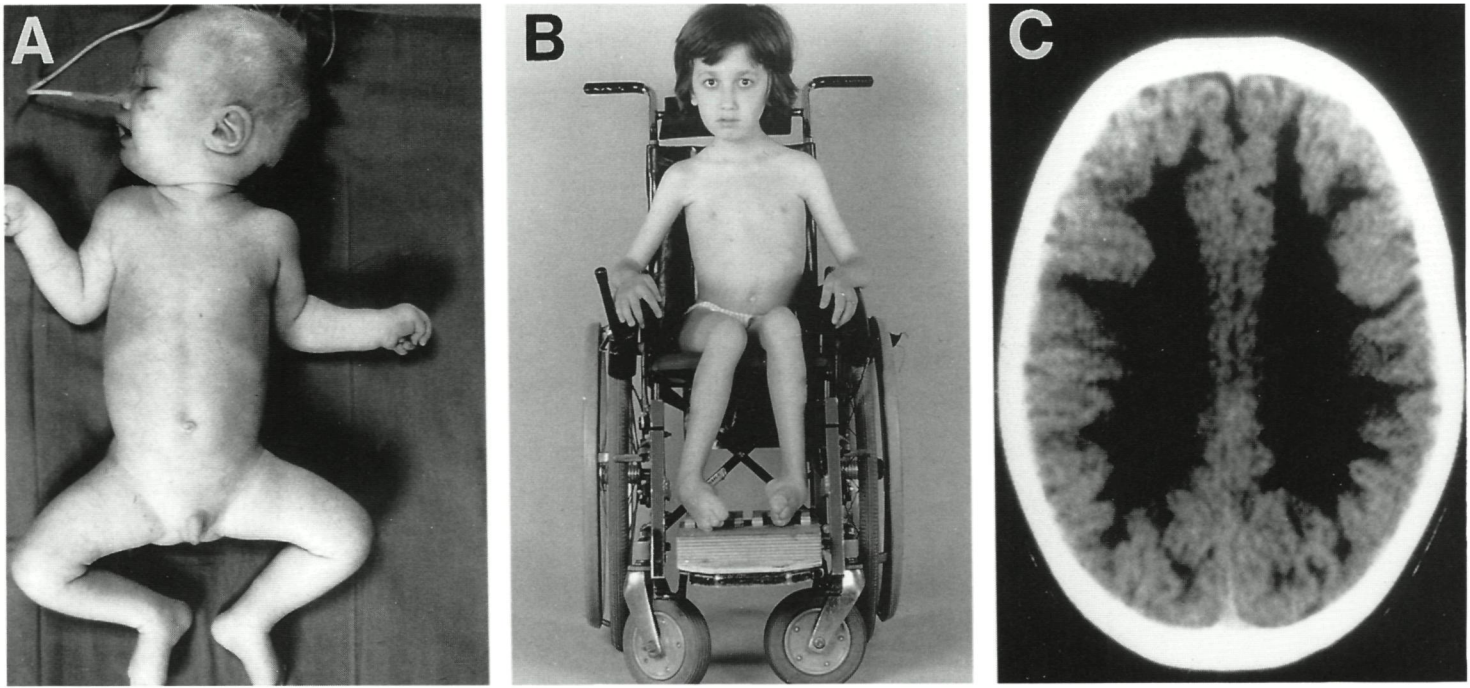

Fig. 2. (A) Infant with the 'pure' form of CMD and merosin positive muscle biopsy or CMD type IA. (B) Child with the 'pure' form of CMD and merosin negative muscle biopsy or CMD type IB. (C) Cerebral CT with diffuse hypodensity of white matter in CMD type IB.

Neurophysiological examination

In most cases, electromyographic (EMG) studies indicate a myopathic disorder with an increased number of brief polyphasic potentials with a small amplitude Motor nerve conduction velocities (MNCVs) are normal in all patients Electroencephalography (EEG) and somatosensory evoked potentials (SEPs) [69] are normal

Radiological examination

Cerebral CT and/or MRI scans do not reveal any abnormalities

Morphological aspects

Muscle biopsy Histological abnormalities of muscle biopsy specimens are variable and usually include increased muscle fibre diameters, an increase in endomysial and perimysial fat and connective tissue (Fig 1A), and some regeneration [66,67,70-72] Fibres are rounded and fibre necrosis, fibre splitting, phagocytosis, predominance of type I or type II fibres, selective hypertrophy and selective atrophy have been seen, but not consistently These dystrophic changes generally depend on the stage of the disorder and are usually progressive Morphological follow-up studies on individual cases with CMD have not been done until now

Immunohistological staining with antibodies against laminin A, B1, B2, M, collagen IV, spectrin and dystrophin (Fig 1B) [76-79] and dystrophin-associated glycoproteins [79] are normal or near-normal Merosin (merosin M-chain or laminin-α_2) is a striated muscle specific basal lamina associated protein The locus for the merosin gene is on chromosome 6q2 [70,80] Mercuri et al studied the muscle merosin (merosin M-chain or laminin-α_2) status in 10 'pure' CMD patients without any white matter hypodensity [69] All had normal merosin expression (Fig 1C)

Genetics

There are sets of familial cases The disease may appear sporadically or be transmitted as an autosomal recessive trait [67,70-72] Only one publication on a family with CMD and minimal CNS involvement (i e seizures and poor reading ability in the mother, and an abnormal EEG in the son) suggests an autosomal dominant pattern of inheritance [81] Therefore autosomal dominant or X-linked inheritance is possible in this family

CMD IB. Classic or 'pure' form of congenital muscular dystrophy without severe impairment of intellectual development, with white matter hypodensity and deficient merosin (merosin M-chain or laminin-α_2) status [82,83]

Signs, symptoms and clinical course

The clinical features are -in general- similar to those in CMD patients without any white matter hypodensity, except for (1) usually more severe clinical expression (including subnormal intelligence) and course and (2) some striking dysmorphic features, such as a large head, long and thin face, high arched palate and abnormalities of jaw articulation [58,66] Joint contractures are observed twice as frequently in CMD with white matter hypodensity than in patients with CMD without white matter hypodensity (Fig 2B) [66] In the literature [37,40, 54-67], the occurrence of epilepsy was mentioned in 13 out of 64 patients A recent study demonstrated that perceptuo-motor difficulties and minor neurological soft signs were a consistent feature in CMD children with diffuse MRI changes but not in those with normal MRI, irrespective of the severity or the extent of the contractures and weakness [84] There are no structural ophthalmological abnormalities

Laboratory findings

CK levels are higher than in CMD without white matter hypodensity [66] No reference was made to findings of demyelination when cerebrospinal fluid (CSF) was examined [65,66]

Neurophysiological examination

EMG studies are similar to those of CMD without white matter hypodensity EEG abnormalities are not uncommon [37,40,54-67] Spikes and slow waves, periodic complexes and focal spikes can be detected in routine EEG recordings [65,66] Patients with white matter changes on MRI have abnormalities in the somatosensory evoked potentials (SEPs) [69] Visual evoked potentials are less sensitive than SEPs for detecting abnormalities in children with white matter changes on MRI [69]

Radiological abnormalities

Cerebral CT and/or MRI indicates marked hypodensity of the white matter (Fig 2C) The severity of this hypodensity differs from case to case Most patients have brain MRI changes of diffuse white matter involvement, resembling

leukodystrophy, usually sparing the internal capsule, basal ganglia, corpus callosum and brain stem [63,65,84]. There is a lack of progression of white matter hypodensities on successive CT/MRI scans [36,59,63], but long-term evaluation of the evolution of the hypodensities is needed.

Morphological aspects

Muscle biopsy. The histological appearance of muscle biopsies from CMD with white matter hypodensity are in general similar to those of CMD without white matter hypodensity, except that more necrosis was seen in 4 patients with CMD with white matter hypodensity [66]. Immunohistological staining with antibodies against laminin A, B1, B2, collagen IV, spectrin, dystrophin [69,76-78] and dystrophin-associated glycoproteins [78] were normal. In 1995, Mercuri et al. [69] described 8 patients with CMD and abnormal cerebral MRI who all had a negative merosin status (Fig. 1D), in contrast with 10 patients with 'pure' CMD and normal cerebral MRI.

Neuropathological examination of the brain. The nature and significance of the white matter changes remain obscure. Informative neuropathological data are scarce. Before the introduction of CT/MRI, some authors described demyelination in the brain in CMD [32]. There is only one report, in which two patients are described [36]. One patient showed moderate focal subpial gliosis and good preservation of the cortical architecture in a brain biopsy from the right frontal lobe. The myelin sheaths were intact in the core of the convolution. In the white matter, there was obvious astrocyte proliferation. In the other patient, microscopic examination showed patchy demyelination of the white matter of the centrum semiovale. The authors suggested that these neuropathological findings in themselves were not diagnostic, but that low density in the central areas of the brain on CT/MRI scans may indicate a demyelinating process. In one other case, the white matter had a spongy appearance on necropsy [56]. Malik et al. described a case with significantly decreased staining of myelin compared to age-matched, normal control brains [85]. From these few studies, no definitive conclusions can be drawn as to whether the neuropathology of the white matter hypodensity is caused by de-, hypo- or dysmyelination. In all these patients, a normal cortical gyration was found. Recently, Voit et al. [86] postulated that the white matter hypodensities on CT/MRI are characteric of dysmyelination of the central nervous system.

Genetics

The disease may appear sporadically or be transmitted as an autosomal recessive trait [67,70-72]. Recent studies suggest that in 'pure' CMD the cases with white matter changes on CT/MRI are all merosin-negative [69,84]. The merosin (merosin M-chain or laminin-α_2) is linked to the LAMA 2 gene on chromosome 6q2 [80,82,83], but no mutations of this gene in humans have been reported so far [80]. Until now, no direct relation has been demonstrated between the hypodensities of the white matter and the merosin (merosin M-chain or laminin-α_2) deficient muscle status.

CMD II. Fukuyama type CMD (F-CMD) with structural brain abnormalities and associated mental retardation

F-CMD consists of severe congenital muscular dystrophy, mental retardation and mild cobblestone (previously type II) lissencephaly.

Signs, symptoms and clinical course [30,31,67,70]

A high percentage (28.5%) of the mothers of patients with Fukuyama congenital muscular dystrophy have had spontaneous abortions. The mothers are often aware that fetal movements of affected infants are diminished. At birth, a number of clinical features are apparent. Weakness is generalized, but proximal muscles are affected more than distal ones. The infant sucks poorly and the cry is weak. The face lacks expression and the infant is usually hypotonic (Fig. 3). A funnel chest deformity is noted in about 30%. Pseudohypertrophy of muscle, particularly of the gastrocnemii, is seen in about half of the cases. Mild contractures at the knees and elbows are often present or gradually develop and the tendon reflexes are decreased or absent. Motor development is greatly retarded; the infant supports his or her head poorly and is unable to sit up until almost one year of age. Although many children are able to sit and to crawl, only a small percentage can stand at 4 years of age. The distribution of the affected regions is generalized. Not much difference is found between the proximal and distal muscles or between the shoulder and hip girdles, but the proximal muscles are slightly more severely affected. Contractures at the knees, elbows and hips gradually develop. Exceptionally the child is able to take a few steps. Along with weakness of the facial musculature, the mouth assumes an inverted V-shape. By 8 to 10 years of age, the limb muscles are atrophic and virtually paralyzed. The majority of these children die by 10 years of age. There is a high

association with seizures (37% have febrile seizures and 20% have persistent epilepsy). The most common type of seizure is a major, generalized tonic-clonic convulsion. Status epilepticus occurs occasionally and may result in death. During the first few years of life, it becomes evident that intellectual development is also retarded. The child is unable to form sentences and can understand only the simplest commands. However, in Fukuyama's personal series of 83 cases, the IQ varied from 20 to 90, which indicates that the intellectual deficit is not always severe. One of the clinical aspects that distinguishes the Fukuyama type CMD from the Finnish type of muscle-eye-brain disease and the Walker-Warburg syndrome, is the absence of any major ocular involvement, although there are some reports of ocular abnormalities [31,67]. About half of the patients have minor eye anomalies, such as strabismus, abnormal eye movements or mild myopia. A few have severe myopia, cataracts, optic disc pallor or irregular greyish mottling of the retinal periphery [87]. Some patients have other minor congenital anomalies, such as brachycephaly, a high arched palate, hypertrichosis and protruding heels [88].

Fig. 3. Infant with Fukuyama type of CMD (F-CMD) or CMD type II.

Prevalence. In Japan, the ratio of the Fukuyama form of CMD to Duchenne dystrophy is 1 : 2.1 [31]. Takeshita et al. [89] estimated that 1 in every 18,000 Japanese children between the age of 5 and 19 years is affected by Fukuyama congenital muscular dystrophy. In countries other than Japan, Fukuyama CMD is very rare [32-41]. Dobyns et al. [88] believe that most non-Japanese patients diagnosed as having Fukuyama type of CMD have more severe anomalies typical of the Finnish type of muscle-eye-brain disease (F-MEB-D) or Walker-Warburg syndrome (WWS).

Laboratory findings

Serum CK level is generally higher than in 'pure' CMD, but may decline from the age of 6 years. Also serum GOT, GPT, LDH and aldolase activities are found to be higher [31,67]. In CSF, no evidence has been found to indicate possible cell loss or demyelination [90].

Neurophysiological examination

In most cases, EMG studies indicate a myopathic disorder with an increased number of brief polyphasic potentials with a small amplitude. EEGs often show focal paroxysmal discharges, abnormal background rhythms and other abnormal findings [30,31,67,70,91]. In infants and younger children, these focal spikes are mainly present in the fronto-centro-parietal region, while in older patients, they are also present in the centro-occipital region. Visual evoked potential (VEP) studies can show low amplitude of N2 waves, or prolongation of the peak latency of N2 waves.

Radiological examination

Cerebral CT/MRI examinations reveal asymmetry of the skull in 77%, mild decreased radiodensity of the white matter in 23% and marked hypodensity in 18%. Some authors found hypomyelination in the temporoparietal and frontal white matter. This hypomyelination usually diminished at around one year of age and normalized as the child grew older [90,92]. There is dilatation of the lateral ventricles (59%), third ventricle (41%), fourth ventricle (28%), cortical sulci (50%), longitudinal cerebral fissure (45%), Sylvian fissure (91%) and incisura cerebelli posterior (25%). None of the patients showed cavum septi pellucidi [31]. Another characteristic revealed by MRI study is the presence of pachygyria [90,92].

Morphological aspects

Muscle biopsy Fukuyama et al [31] described the spectrum of muscle abnormalities as follows In the skeletal muscle, advanced connective tissue infiltration into the endomysium and perimysium enlarges the interfibrillar space and the basic structure of the fibre bundle is destroyed Generally, fat infiltration tends to be less severe than connective tissue infiltration In the latter period of the pathological change, only small round fibres remain as islands or are scattered in the midst of a huge amount of connective tissue and fat Within each muscle fasciculus there is striking variation in fibre diameter Less hypertrophic fibres are found, with small-sized fibres being predominant Muscle fibres are observed in varying stages of necrosis Ultrastructural studies have not shown any specific identifying features [93] Some authors observed mild focal cellular infiltrates in the perimysium and endomysium as well as around blood vessels [94,95]

In most cases, dystrophin distribution is normal or near-normal (occasional abnormally immunostained pattern) [76,78,79,96,97], although exceptionally dystrophin-negative patients have been observed (perhaps atypical Duchenne dystrophy cases?) The intensity of immunostaining for muscle-specific merosin (merosin M-chain or laminin-α_2) was shown to be greatly reduced to 26% by quantitative immunofluorescence [78] However, merosin was normally expressed in intrafusal fibres, which suggests a secondary change [78,88] Greatly reduced staining for dystrophin-associated proteins -especially the 43 kDa component (43 DAG)- was found in 12 F-CMD patients [96] However, these proteins were excluded as candidates for the primary defect in F-CMD, because no positive linkages were found with loci in the 6q2 region containing the LAMA 2 gene and in the 3p21 region containing the 43 DAG gene [82,83,98]

Neuromorphological studies on the peripheral nervous system, i e spinal anterior roots and medullated nerve fibres, in F-CMD did not reveal any abnormalities [99,100]

Neuropathological examination of the brain [30,31,88,101,102] Cobblestone lissencephaly (previously type II lissencephaly) is a complex brain malformation which consists of cobblestone cortex, abnormal white matter, enlarged ventricles, small brain stem and small cerebellum, especially vermis with cerebellar polymicrogyria The descriptive term 'cobblestone cortex' was proposed by Haltia [67,70,88] The cortical changes consist of mixed agyria, pachygyria and polymicrogyria with a pebbled surface The spectrum begins with widespread agyria (but with a pebbled surface) and probably ends with mixed frontal pachygyria and posterior polymicrogyria Leptomeningeal neuronal and glial

heterotopia partly obstruct the subarachnoid space and may fuse the hemispheres. The white matter changes vary from simple myelin pallor to severe oedema and cystic degeneration [88].

F-CMD consists of cobblestone lissencephaly which is less severe than in F-MEB-D or WWS [31,88,103]. The cobblestone cortex consists of pachygyria and polymicrogyria with partial obstruction of the subarachnoid space and hemispheric fusion. Other abnormalities include white matter abnormalities which improve with age, mildly enlarged ventricles, mild cerebellar polymicrogyria and hypoplasia of the pyramidal tracts. However, the majority of cases did not show any remarkable abnormalities in the cerebral white matter [101,102]. Although the neuropathological changes are consistent with the loss of normal cortical cytoarchitecture from a histological point of view, the distribution and the degree of the pathological changes can differ considerably from one case to another [30,31,101,102].

Genetics

F-CMD (or CMD type II) is transmitted as an autosomal recessive trait [30,31]. A 25% incidence of consanguineous marriages is found within these families. The sexes are almost equally affected and there is a high frequency of affected siblings. The gene responsible for the F-CMD has recently been localized on chromosome 9q31-33 [98].

CMD III Finnish type of muscle-eye-brain disease (F-MEB-D)

F-MEB-D consists of congenital muscular dystrophy, mental retardation, retinal hypoplasia and cobblestone lissencephaly [42,43,65, 67,70,88,104].

Signs, symptoms and clinical course [42,43,65,67,70,104]

Clinical manifestations include muscle weakness, hypotonia (often associated with distal spasticity) and ocular abnormalities, especially retinal hypoplasia. Muscle weakness is generalized, affecting especially the muscles of the trunk and the extremities. Muscular dystrophy appears before the age of 1 year. The head lag is extremely severe. In the extremities, muscle weakness is slightly more prominent in the proximal muscles. After the age of 5 years, slight spasticity develops in many patients especially in the legs, but can be difficult to recognize later on because of contractures. All the patients have weak or absent

reflexes until they develop spasticity, when brisk reflexes can again be found before the development of contractures Most patients have poor vision Eye signs are high myopia, congenital or infantile glaucoma associated with angle abnormalities, iris hypoplasia, cataracts, optic nerve pallor, choroidal hypoplasia, colobomas and retinal atrophy The eyes are hydrophthalmic during infancy in patients with glaucoma and become deepset later on Microphthalmia, typical retinal dysplasia and corneal opacity have not been observed All cases are mentally retarded, most of them severely but some only moderately, 10 out of the 20 (personal experience of Santavuori) achieved some speech Epilepsy is a common associated feature The facial appearance shows some similarities in most patients The head appears large with a high and prominent forehead and wide fontanelle The calvarium is narrow in the temporal region The palate is always narrow The midfacies is flat and the nose and the philtrum appear short The head size remains on the +1 to +2 SD curve in some patients after the age of 15 years, but is ultimately smaller than average in most cases Usually the head circumference grows too rapidly during the first year of life and a tense fontanelle is seen Although some die in childhood, most survive to 10-30 years and some even longer

Laboratory findings

CK values are elevated in all the patients, but some are normal during the first year, reaching the highest values between 5 and 15 years of age Examination of the CSF does not reveal any abnormalities [65]

Neurophysiological examination

The EMG can be normal initially, but after 1 to 2 years of age, it always shows a typical myopathic pattern with small and polyphasic motor unit potentials During the first 6 months of life, the EEG is normal but after the age of one year it becomes abnormal with a slow and irregular background, attenuated activity in the posterior regions, an excess of beta activity and exaggerated spindles in sleep Paroxysmal activity is found in half of the recordings During early infancy, the ERG may be normal, but after the age of 1 year, the responses are absent or severely attenuated Visual evoked potentials (VEPs) are also normal during infancy, but usually change to unusual giant potentials after the age of 1-2 years The combination of low values on the electroretinogram and very high visual evoked potentials in most patients is a striking but not specific feature, because it has also been described in late infantile ceroid lipofuscinosis [105,106]

Radiological examination

Neuroimaging demonstrates enlargement of the lateral ventricles and fourth ventricle In some patients, the third ventricle is also enlarged, especially at the anterior part Also cortical atrophy can be present [104] There is -patchy- low density white matter in about half of the patients, while the white matter can appear quite normal in others, even on some high-quality MRI scans Examination of the brain of 10 patients with F-MEB-D using high-field MRI revealed a uniform pattern consisting of pachygyria, septal and corpus callosum defects and severe hypoplasia of the pons in 7 of them [107]. Some patients may have progressive hydrocephalus, apparent hydranencephaly, Dandy-Walker malformation and occipital cephaloceles [65,88].

Morphological aspects

Muscle histology Muscle biopsy tissue obtained from patients of various ages (from a few months to 56 years) showed histological findings that varied from nearly normal to severely dystrophic. Muscular dystrophy appears before the age of 1 year. An increased number of internal nuclei and abnormal variation in the fibre size is common. There are more atrophic fibres than hypertrophic ones. Fibre splitting is also seen in a number of patients, but necrotic and regenerative fibres are rare. In the most severely affected patients, increased endo- and perimysial fat and connective tissue are obvious. The severity of the changes increases with age, but the histological picture of burned-out myopathy is not seen even in the oldest patients [104]. No reports have appeared on the merosin (merosin M-chain or laminin-α_2) status of F-MEB-D patients.

Neuropathological examination of the brain. Autopsy was performed on two patients with Finnish type MEB-D who died at the age of 15 and 34 years, respectively Both brains showed a global abnormality in the gyral pattern. The surface was covered by small nodules, the so-called 'cobblestone cortex', with pachygyria over the frontal region and polymicrogyria posteriorly. Histologically, there was disruption of the normal cortical cytoarchitecture, with loss of the regular boundary between the neuroectodermal tissue and the pia arachnoid. The cerebellum showed the presence of the various normal elements but in a haphazard arrangement, comparable to a 'scrambled egg' [67]. Pihko described a severely mentally retarded boy with occipital encephalocele and a Dandy-Walker cyst [65].

Pathological examination of the eyes. Eye pathology includes the loss of ganglion cells and a thick abnormal preretinal layer, abnormal retinal pigment epithelium, Peter's anomaly, anterior chamber malformations, cataract, persistent

hyperplastic primary vitreous (PHPV), abnormal vascularization and hypoplasia of the optic nerve [54,67,108-110].

Genetics

Muscle-eye-brain disease has an autosomal recessive mode of inheritance. Recently, Ranta et al. demonstrated that Finnish type MEB-D and F-CMD are not allelic [111]. They excluded the F-MEB-D gene from the region of the F-CMD gene on chromosome 9q31-33. The gene responsible for the Finnish type of MEB-D has not yet been detected.

CMD IV Walker-Warburg syndrome (WWS)

WWS consists of congenital muscular dystrophy, severe mental retardation, retinal abnormalities and severe cobblestone lissencephaly [44-53,86,88]

Signs, symptoms and clinical course [44-53,86,88]

Pregnancy is often complicated by polyhydramnios and decreased fetal movements. Delivery is often by Caesarian section because of fetal distress and/or cephalopelvic disproportion due to an enlarged fetal head. Many patients are stillborn or die during the perinatal period. The mean survival is only four months, although some patients may survive for more than five years [88].

Most children have severe or profound mental retardation, hypotonia and muscle weakness, mild distal spasticity and often poor vision. Muscular dystrophy appears before the age of 1 year. A few patients have only moderate mental retardation and are able to roll over and sit up. Seizures may occur during the neonatal period and are common at older ages; treatment may be difficult [52]. Congenital macrocephaly is due to hydrocephalus, but microcephaly is also encountered. The eye abnormalities consist of microphthalmia (Fig. 4A), iris coloboma, buphthalmos, glaucoma, Peter's anomaly, cataract, corneal opacities in the anterior chamber, microphthalmia, hypoplasia of the optic nerve, typical retinal dysplasia and detachment, vitreous haemorrhage and persistent vessels. Many other congenital anomalies have been reported in WWS patients: adrenal hypoplasia, cleft lip/palate, urogenital anomalies in males, hearing loss, renal dysplasia/hypoplasia [44-53,86,88].

Laboratory findings

All the patients showed an increased serum CK level, but there was great variation between the patients (3 to 60 times the upper limit of normal) or over time in an individual patient [52].

Neurophysiological examination

EMG studies produced a severe myopathic trace, while motor nerve conduction velocities were normal [52,67,70]. EEG showed diffuse slowing and paroxysmal discharges. Visual evoked potentials (VEPs) and brainstem auditory evoked potentials (BAEPs) appeared to be severely disturbed.

Radiological examination

MRI mostly showed changes typical of cobblestone lissencephaly: agyria or pachygyria, usually combined with polymicrogyria, severe and diffuse white matter abnormalities. These consisted of radiolucency on CT scans or a bright signal on T2-weighted MRI, enlarged ventricles often severe enough for hydrocephalus, brain stem hypoplasia, cerebellar hypoplasia, especially vermis hypoplasia, with Dandy-Walker malformations, while one third of the patients had occipital encephalocele or meningocele (Fig. 4B) [50,52,67,70,88].

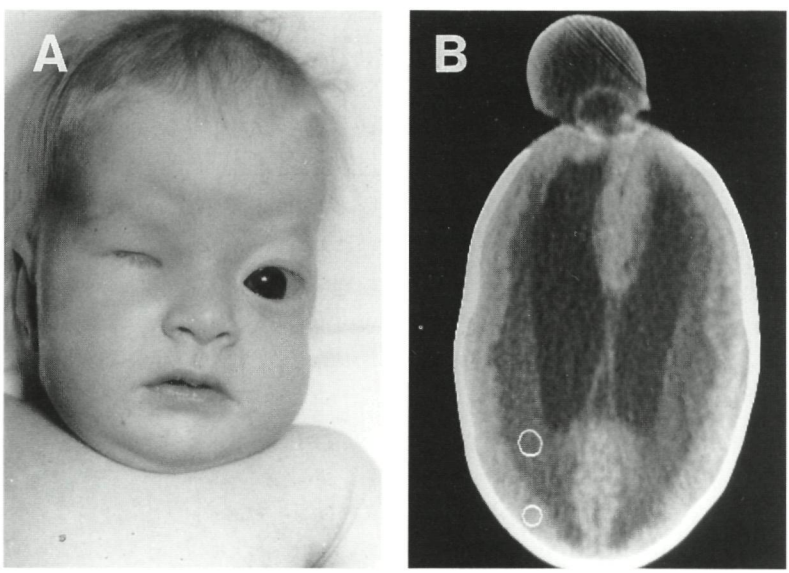

Fig. 4. (A) Child with the Walker-Warburg type of muscle-eye-brain disease (WWS) or CMD type IV. (B) Cerebral CT illustrating occipital encephalocele and ventricle dilatation in WWS (CMD type IV).

Morphological aspects

Muscle histology. The histological and histochemical features are consistent with congenital muscular dystrophy [52]. CMD is often diagnosed before the age of 1 year. The pathological changes can vary in severity between muscles and even between fascicles [52]. In some patients, the muscle changes are less severe and are then classified as unspecific myopathy. In a recent study, preserved merosin (merosin M-chain or laminin-α_2) was reported consistently in 5 patients with WWS [86].

Neuropathological examination of the brain. Severe 'cobblestone cortex' is present in all patients [67]. The gross appearance is dominated by widespread agyria with scattered areas of pachygyria and/or polymicrogyria [52]. The leptomeninges are thick and granular and may obliterate the interhemispheric fissure, effectively 'fusing' the cerebral hemispheres. Ventricles are enlarged. The cortex is abnormally thick with absent white matter interdigitations. The white matter is oedematous and sometimes shows cystic degeneration. The corpus callosum and septum pellucidum are very frequently absent or hypoplastic. Microscopically, the subarachnoid space is partially occluded by fibrous and heterotopic neuroglial tissue. The cortex is severely disorganized with no recognizable layers and widespread disruption by abnormal vascular channels and fibrous glial bands. The white matter is poorly myelinated with scattered heterotopic neurons.

Cerebellar malformations comprise polymicrogyric or smooth (afoliar) surfaces, hemispheric and especially vermis hypoplasia and characteristic microscopic changes reminiscent of those in the cerebrum. However, some cortical layers can usually be recognized and the white matter abnormalities are less severe. The vermis, especially the posterior vermis, is always hypoplastic. This is often associated with enlargement of the fourth ventricle to form a retrocerebellar cyst, usually referred to as Dandy-Walker malformation.

Occipital cephaloceles, meningoceles and encephaloceles, have been described. In most cases, a cephalocele represents an extension of a retrocerebellar cyst through an occipital skull defect [50,52,67,70,88].

Pathological examination of the eyes. The ophthalmological signs include severe structural eye changes, such as microphthalmia and colobomas, cleavage defects of the anterior chamber resulting in congenital glaucoma, optic cup deformation resulting in severe myopia, cataract and retinal detachment secondary to retinal dysplasia. Also persistent hyperplastic primary vitreous (PHPV), retinal pigment epithelium abnormalities and Peter's anomaly have been described.

Histologically, the inner retina is thin with abnormal gliovascular proliferation. The ganglion cells are decreased in number and poorly differentiated with

few axons and dendrites. The nerve fibre layer is secondarily thin or absent, resulting in small (hypoplastic) or absent optic nerves. Photoreceptors are present, but their outer segments are poorly formed or absent. There may be areas of abnormal differentiation with retinal folds and rosettes. The retinal pigment epithelium is usually irregularly pigmented and may have areas of hyperplasia with drusen formation. In the most severe cases, there is so-called PHPV in which the retina forms a mass of gliotic tissue extending from the posterior pole to the posterior surface of the lens. Microphthalmia commonly accompanies PHPV [52].

Genetics

An autosomal recessive mode of inheritance is more or less certain on the grounds of the following observations: (1) normal sex ratio in affected children, (2) several families have multiple affected children of both sexes, (3) healthy parents and (4) several instances of parental consanguinity. In the review by Dobyns et al. [52], nine out of twenty-five sibs (36%) were affected. Parental consanguinity was documented in one of the twenty-eight families in the literature (third-cousins) [112] and in two out of nineteen families in the series described by Dobyns et al. [52]. No ethnic, racial, or geographic predilections are apparent [52].

Toda et al. [113] claimed genetic identity of F-CMD and WWS. Their findings were based on microsatellite marker analysis of two siblings: one presumed to be suffering from F-CMD and one from WWS. Both showed the same alleles for the flanking markers of the F-CMD locus on 9q31-33. However, no statistical analysis or lod score calculation were provided and no mutation of the F-CMD gene was demonstrated. Various explanations can account for this observation [86].

Comparison between F-CMD, F-MEB-D and WWS (Table 3)

While Fukuyama type CMD has clinical and neuropathological similarities with F-MEB-D and WWS concerning autosomal recessive inheritance, dystrophic changes in muscle, pachygyria or polymicrogyria of the brain and sometimes eye abnormalities, it has long been considered as distinct from F-MEB-D and WWS. The reasons are as follows: (1) the spectrum of gyral abnormalities is much less severe and does not typically extend to complete or near-complete

agyria, (2) there is a lack of major eye abnormalities, (3) major cerebellar abnormalities are rare [30,31,52,67,70] and (4) the immunostaining intensity of merosin (merosin M-chain or laminin-α_2) is significantly reduced in F-CMD [78], but (in contrast) merosin expression in WWS is consistently preserved [86]. Yoshioka et al. [114] observed two sibs with discordant manifestations. The younger sib had severe WWS while the older had F-CMD. However, after reviewing the brain and eye abnormalities, Dobyns et al. [88] suggested that the latter actually had F-MEB-D or mild WWS. Toda et al. recently mentioned that F-CMD and WWS may in some cases be due to variable expression of the same genetic defect [113], but no statistical analysis or lod score calculation were provided and no mutation of the F-CMD gene was demonstrated [86]. Various explanations can account for this observation, e.g. a mutation of the F-CMD gene leading to unusually severe phenotypes, a combination of different mutations present in one family, etc. [86].

The consistently preserved merosin (merosin M-chain or laminin-α_2) expression in the skeletal muscle of 5 (all) WWS patients is in contrast with F-CMD [86]. Therefore, some authors believe that merosin can be used as an immunocytochemical marker to distinguish WWS from F-CMD [86]. However, F-CMD (with reduced merosin expression) could theoretically be caused by different mutations of the same gene. Ultimately, this issue can only be solved by linkage and subsequent gene analysis [86]. Moreover, Ranta et al. demonstrated that F-CMD and F-MEB-D are not allelic [111].

At present, there is considerable discussion among the consortium participants of the two ENMC sponsored International Workshops on CMD [67,70], as to whether F-MEB-D and WWS should be considered as one entity. Initially, muscular dystrophy was not detected in WWS; the muscle disease was probably overshadowed by the severe cerebro-ocular abnormalities. Later, however, Dobyns et al. [70] made a detailed comparison between F-MEB-D patients from Finland and WWS patients from the United States and elsewhere (Table 4). At the workshop, comparative MRI/CT studies on 20 patients with F-MEB-D or WWS demonstrated striking similarities [70]. Nevertheless, there were quantitative differences between the F-MEB-D and WWS patients (Tables 3 and 4). In F-MEB-D patients, the gyral malformation most often consisted of pachygyria over the frontal region and polymicrogyria posteriorly. The white matter changes were present but patchy in about half of the F-MEB-D patients, while the white

Table 3. Comparison of F-CMD, F-MEB-D and WWS (adapted from Dobyns [52,88])

	F-CMD	F-MEB-D	WWS
Chromosome localization of responsible gene	9q31-33	?	?
Cobblestone (type II) lissencephaly	+++	+++	+++
Predominate agyria	0	0	+++
Predominate pachygyria	++	+++ (frontal)	++
Predominate polymicrogyria	++	+++ (posterior)	+
White matter lucency	50% improving with age	50% (mild, patchy)	+++ (severe, diffuse)
Cerebellar malformations	+++	+++	+++
Cortical dysplasia	+++	+++	+++
Vermis hypoplasia	0	++	+++
Dandy-Walker malformation	0	±	++
Occipital cele	0	±	++ (30%)
Eye abnormalities	±	+++	+++
Typical retinal dysplasia	0	0	++
Optic nerve pallor	±	++	+++
Other retinal abnormality	±	+++	++
Anterior chamber malformation	±	++	++
Persistent vessels	0	+	++
Microphthalmia	0	0	++
Muscle disease			
CMD	+++	+++	+++
Calf pseudohypertrophy	+	0	0
Merosin reduced	++	?	0
Associated abnormalities			
Ventricular dilatation	++ (mild)	++	+++
Hypoplastic corpus callosum	+	++	++
Brain stem hypoplasia	0	+	+++
Congenital macrocephaly	0	+	++
Congenital microcephaly	++	±	±

+++ = constant, ++ = frequent, + = occasional, ± = very rare, ? = unknown, 0 = not observed.

Table 4. Differences between Finnish type of muscle-eye-brain disease (F-MEB-D) and Walker-Warburg syndrome (WWS), modified according to Dobyns [52,88].

F-MEB-D	WWS
Mental retardation; mild to severe	Mental retardation; always severe
Structural eye abnormalities	Structural eye abnormalities
a) absent microphthalmia	a) microphthalmia
b) absent typical retinal dysplasia	b) typical retinal dysplasia
c) absent corneal opacities	c) corneal opacities
Cobblestone lissencephaly with	Cobblestone lissencephaly with
a) primarily frontal pachygyria and occipital polymicrogyria	a) agyria with pachygyria and polymicrogyria
b) absent or patchy white matter changes	b) diffuse white matter changes; cortex lacks white matter interdigitations
c) very rare occipital cele	c) frequent occipital cele

matter appeared to be quite normal in the others, even in some with high-quality MRI scans. Most WWS patients had severe gyral changes that consisted of agyria or mixed agyria-pachygyria with some areas of polymicrogyria. All the WWS patients had severe and diffuse white matter changes and severe to profound mental retardation, which made them more severely handicapped than most of the F-MEB-D patients [70]. All the differences appeared to be quantitative, especially the severity of the gyral malformation and the white matter changes. The cerebellar malformations may have been somewhat more severe in WWS. This is supported by the frequency of occipital celes, which occur in about 30% of patients with WWS but have only been reported sporadically in patients with F-MEB-D. The eye abnormalities in the two disorders also differ quantitatively. However, microphthalmia, typical retinal dysplasia and corneal opacities have not been observed in F-MEB-D [88]. According to Dobyns et al., the similarities far outweigh the differences, making it likely that WWS and F-MEB-D are allelic [88]. Thus, the question must be left open as to whether WWS and the clinically milder but phenotypically similar F-MEB-D are allelic disorders [86,88,115].

In conclusion, the classification of CMD as presented in Table 1 is the most actual, but it is possible that this classification will be changed after new genetic research findings become available. For definite delineation of the nosological entities of CMD, further molecular genetic research is necessary.

References

[1] Oppenheim, H. (1900) Über allgemeine und lokalisierte Atonie der Muskulatur (Myotonie) im frühen Kindesalter. Monatsschr. Psychiatr. Neurol., 8: 232.

[2] Brandt, S. (1950) Werdnig-Hoffmann's infantile progressive muscular atrophy. Kopenhagen: Munksgaard.

[3] Arthuis, M.M.P. (1954) L'amyotrophie spinale infantile de Werdnig-Hoffmann. Paris, Thèse.

[4] Batten, F.E. (1903) Case of myositis fibrosa with pathological examination. Trans. Clin. Soc. Lond., 37: 12.

[5] Howard, R. (1908) A case of congenital defect of the muscular system (dystrophia muscularis congenita) and its association with congenital talipes equinovarus. Proc. R. Soc. Med., 1: 157-166.

[6] Lereboullet, M.M., Baudouin, A. (1909) Un cas de myatonie congenitale avec autopsie. Bull. Soc. Med. Hôp. Paris, 3: 1162.

[7] Councilman, W.T., Dunn, C.H. (1911) Myatonia congenita: a report of a case with autopsy. Am. J. Dis. Child., 2: 340.

[8]. Haushalter, P. (1920) Sur la myatonie congénitale (maladie d'Oppenheim). Arch. Med. Enf., 23: 133.

[9] Ullrich, O. (1930) Kongenitale, atonisch-sklerotische Muskeldystrophie, ein weiterer Typus der heredodegenerativen Erkrankungen des neuromuskulären Systems. Z. Ges. Neurol. Psychiatr., 126: 171-201.

[10] Ullrich, O. (1930) Kongenitale, atonisch-sklerotische Muskeldystrophie. Monatsschr. Kinderheilkd., 47: 502-510.

[11] Stoeber, E. (1939) Über atonisch-sklerotische Muskeldystrophie (Typ Ullrich). Z. Kinderheilkd., 60: 279-284.

[12] Gött, H., Josten, E.A. (1954) Beitrag zur kongenitalen atonisch-sklerotischen Muskeldystrophie (Typ Ullrich). Monatsschr. Kinderheilkd., 75: 105-118.

[13] Schneider, H. (1957) Die atonisch-sklerotische Muskeldystrophie (Ullrich) im Rahmen mesodermaler Dysplasien. Z. Orthop., 88: 397-404.

[14] Joh, M. (1976) A case of Ullrich syndrome. Clin. Neurol. (Tokyo), 16: 728.

[15] Naito, M., Okada, R., Segawa, M. (1974) A case of Ullrich disease. Clin. Neurol. (Tokyo), 14: 813.

[16] Nakano, Y. (1976) A case of Ullrich syndrome. Clin. Neurol. (Tokyo), 16: 597.

[17] Nonaka, I, Ueno, T , Miyoshino, S , et al. (1974) Clinical and pathological study of Ullrich type of congenital muscular dystrophy Brain Dev (Tokyo) (Domestic ed.), 6: 45-56.

[18] Sato, A , Sannomiya, A (1970) A case of Ullrich syndrome. Clin Paediatr., 10 1261-1263.

[19] Segawa, M., Lin, K., Kawaguchi, H. (1970) A case of Ullrich disease. Clin Neurol (Tokyo), 9 513.

[20] Toyokura, Y., Takasu, T , Yanagisawa, N (1966) Kongenitale atonisch-sklerotische Muskeldystrophie (Typ Ullrich). Clin. Neurol (Tokyo), 6 742

[21] Nihei, K., Kamoshita, S., Atsumi, T. (1979) A case of Ullrich's disease (kongenitale, atonisch-sklerotische Muskeldystrophie). Brain Dev., 1: 61-67

[22] Walton, J N., Natrass, F.J. (1954) On the classification, natural history and treatment of the myopathies Brain, 77 169-231.

[23] Banker, B Q , Victor, M., Adams, R D. (1957) Arthrogryposis multiplex due to congenital muscular dystrophy Brain, 80 319-334.

[24] Pearson, C M., Fowler, W.G. Jr. (1963) Hereditary non-progressive muscular dystrophy including arthrogryposis syndrome. Brain, 86· 75-88.

[25] Adams, R D., Denny-Brown, D., Pearson, C.M. (1962) Diseases of muscle: a study in pathology, 2nd ed Harper, New York, 1962

[26] Drachman, D B., Banker, B.Q. (1961) Arthrogryposis multiplex congenita: case due to disease of the anterior horn cells. Arch. Neurol , 5 77-93.

[27] Drachman, D B , Coulombre, A.J. (1962) Experimental clubfoot and arthrogryposis multiplex congenita. Lancet, 2: 523-526.

[28] Frischknecht, W., Bianchi, L., Pilleri, G. (1960) Familiare Arthrogryposis multiplex congenita· neuro-arthro-myodysplasia congenita Helv Paediatr Acta, 15: 259.

[29] Vestermark, B. (1966) Arthrogryposis multiplex congenita: a case of neurogenic origin. Acta Paediatr. Scand., 55 117-120.

[30] Fukuyama, Y., Kawazura, M., Haruna, H. (1960) A peculiar form of congenital progressive muscular dystrophy: report of fifteen cases. Pediatr. Univ. Tokyo (Tokyo), 4:5-8.

[31] Fukuyama, Y , Osawa, M., Suzuki, H. (1981) Congenital progressive muscular dystrophy of the Fukuyama type: clinical, genetic and pathological considerations. Brain Dev., 3: 1-29.

[32] Fowler, M , Manson, J.T. (1973) Congenital muscular dystrophy with malformation of central nervous system. In: B.A. Kakulas (Ed.), Clinical

studies in myology. Proceedings of the 2nd International Congress of Muscle Diseases, Perth, 1971, Excerpta Medica, ICS 295,. Amsterdam, pp. 192-197

[33] Krijgsman, J.B., Barth, P.G., Stam, F C., Slooff, J.L., Jaspar H.H.J. (1980) Congenital muscular dystrophy and cerebral dysgenesis in a Dutch family. Neuropadiatrie, 11: 108-120.

[34] Nonaka, I., Chou, S M. (1979) Congenital muscular dystrophy. In. P.J. Vinken, G.W Bruyn (Eds.), Handbook of clinical neurology, Vol. 41, Elsevier North-Holland, Amsterdam, pp 27-50.

[35] Warter, J.M., Marescaux, C , Coquillat, G , Walter, P., Micheletti, G., Rohmer, F. (1981) Syndrome autosomique recessif associant myopathie et atteinte du système nerveux central et périphérique. J. Neurol. Sci., 49· 135-151

[36] Egger, J., Kendall, B E., Erdohazi, M., Lake, B.D., Wilson, J., Brett, E.M. (1983) Involvement of the central nervous system in congenital muscular dystrophies. Dev Med. Child Neurol., 25: 32-42.

[37] Echenne, B., Pages, M , Marty-Double, C., Pages, A.M., Astruc, J., Brunel, D. (1982) Progressive muscular dystrophy with central nervous system involvement an original variant of congenital neuromuscular disease [abstract]. Proceedings of the 5th International Congress on Neuromuscular Diseases, Marseille, France, 1982.

[38] Balestrini, M R., Mora, M., Daniel, S. (1982) Congenital muscular dystrophy with central nervous system involvement (Fukuyama disease) [abstract]. Acta Neurol (Napoli), 37: 256.

[39] Martinelli, P., Gabellini, A.S., Ciucci, G., Govoni, E., Vitali, S., Gulli. M R (1987) Congenital muscular dystrophy with central nervous system involvement: case report Eur. Neurol., 26: 17-22

[40] McMenamin, J B , Becker, L.E, Murphy, E.G (1982) Congenital muscular dystrophy. a clinicopathological report of 24 cases. J. Pediatr., 100: 692-697

[41] Olney, K.K., Miller, R G. (1983) Inflammatory infiltration in Fukuyama type congenital muscular dystrophy [letter]. Muscle Nerve, 6: 75-77.

[42] Santavuori, P , Leisti, J., Kruus, S. (1977) Muscle, eye and brain disease: a new syndrome [abstract]. Neuropadiatrie, 8(Suppl): 550.

[43] Raitta, C., Lamminen, M., Santavuori, P., Leisti, J. (1978) Ophthalmological findings in a new syndrome with muscle, eye and brain involvement. Acta Ophthalmol. (Copenh.), 56: 465-472.

[44] Walker, A E (1942) Lissencephaly. Arch. Neurol. Psychiatr., 48: 13-29

[45] Warburg, M. (1978) Hydrocephaly, congenital retinal nonattachment, and congenital falciform fold. Am. J. Ophthalmol., 85: 88-94.

[46] Warburg, M. (1971) The heterogeneity of microphthalmia in the mentally retarded. Birth Defects, 7: 136-154.

[47] Pagon, R.A., Chandler, J.W., Collie, W.R., Clarren, S.K., Moon, J., Minkin, S.A., Hall, J.G. (1978) Hydrocephalus, agyria, retinal dysplasia, encephalocele (HARD +/– E) syndrome: an autosomal recessive condition. Birth Defects, 14: 232-241.

[48] Pagon, R.A., Clarren, S. (1981) HARD +/– E: Warburg's syndrome [letter]. Arch. Neurol., 38: 66.

[49] Pagon, R.A., Clarren, S.K., Milam, D.F. Jr., Hendrickson, A.E. (1983) Autosomal recessive eye and brain anomalies: Warburg syndrome. J. Pediatr., 102: 542-546.

[50] Dobyns, W.B., Kirkpatrick, J.B., Hittner, H.M, Roberts, R.M., Kretzer, F.L. (1985) Syndromes with lissencephaly. II. Walker-Warburg and cerebro-oculo-muscular syndromes and a new syndrome with type II lissencephaly. Am. J. Med. Genet., 22: 157-195.

[51] Towfighi, J., Sassani, J.W., Suzuki, K., Ladda, R.L. (1984) Cerebro-ocular dysplasia-muscular dystrophy (COD-MD) syndrome. Acta Neuropathol. (Berl.), 65: 110-123.

[52] Dobyns, W.B., Pagon, R.A., Armstrong, D., Curry, C.J.R., Greenberg, F., Grix, A., Holmes, L.B., Laxova, R., Michels, V.V., Robinow, M., Zimmerman, R.L. (1989) Diagnostic criteria for Walker-Warburg syndrome. Am. J. Med. Genet., 32: 195-210.

[53] Dobyns, W.B., Pagon, R.A., Curry, C.J.R., Greenberg, F. (1990) Regarding Walker-Warburg syndrome and muscle-eye-brain disease [letter, response to Santavuori et al.]. Am. J. Med. Genet., 36: 373-374.

[54] Bernier, J.-P., Brooke, M.H., Naidich, T.P., Carroll, J.E. (1979) Myoencephalopathy: cerebral hypomyelination revealed by CT scan of the head in a muscle disease. Trans. Am. Neurol. Assoc., 104: 244-246.

[55] Nogen, A.G. (1980) Congenital muscular disease and abnormal findings on computerized tomography. Dev. Med. Child Neurol., 22: 658-663.

[56] Echenne, B., Arthuis, M., Billard, C., Campos-Castello, J., Castel, Y., Dulac, O., Fontan, D., Gauthier, A., Kulakowski, S., De Meuron, G., Moore, J.R., Nieto-Barrera, M., Pages, M., Parain, D., Pavone, L., Ponsot, G. (1986) Congenital muscular dystrophy and cerebral CT scan anomalies: results of a collaborative study of the Société de Neurologie Infantile. J. Neurol. Sci., 75: 7-22.

[57] Castro-Gago, M., Peña-Guitián, J. (1988) Congenital muscular dystrophy of a non-Fukuyama type with characteristic CT images [letter]. Brain. Dev., 10: 60.

[58] Yoshioka, M., Kuroki, S., Mizue, H. (1987) Congenital muscular dystrophy of non-Fukuyama type with characteristic CT images. Brain Dev., 9: 316-318.

[59] Streib, E.W., Lucking, C.H. (1989) Congenital muscular dystrophy with leukoencephalopathy. Eur. Neurol., 29: 211-215.

[60] Tanaka, J., Mimaki, T., Okada, S., Fujimura, H. (1990) Changes in cerebral white matter in a case of congenital muscular dystrophy (non-Fukuyama type). Neuropediatrics, 21: 183-186.

[61] Topaloğlu, H., Yalaz, K., Kale, G., Ergin, M. (1990) Congenital muscular dystrophy with cerebral involvement: report of a case of "occidental type cerebromuscular dystrophy"? Neuropediatrics, 21: 53-54.

[62] Topaloğlu, H., Yalaz, K., Renda, Y., Cağlar, M., Göğüs, S., Kale, G., Gücüyener, K., Nurlu, G. (1991) Occidental type cerebromuscular dystrophy: a report of eleven cases. J. Neurol. Neurosurg. Psychiatry, 54: 226-229.

[63] Cook, J.D., Gascon, G.G., Haider, A., Coates, R., Stigsby, B., Ozand, P.T., Banna, M. (1992) Congenital muscular dystrophy with abnormal radiographic myelin pattern. J. Child Neurol., 7(Suppl): 51-63.

[64] Kaciński, M., Bańdo, B., Kroczka, S., Malinowska-Matuszewska, M.M. (1992) A case of congenital muscular dystrophy with changes in the white matter of the brain (in Polish). Neurol. Neurochir. Pol., 26: 383-387.

[65] Pihko, H., Louhimo, T., Valanne, L., Donner, M. (1992) CNS in congenital muscular dystrophy without mental retardation. Neuropediatrics, 23: 116-122.

[66] Topaloğlu, H., Kale, G., Yalnizoğlu, D., Taşdemir, A.H., Karaduman, A., Topçu, M., Kotiloğlu, E. (1994) Analysis of "pure" congenital muscular dystrophies in thirty-eight cases. How different is the classical type 1 from the occidental type cerebromuscular dystrophy? Neuropediatrics, 25: 94-100.

[67] Dubowitz, V. (1994) Workshop report: 22nd ENMC sponsored workshop on congenital muscular dystrophy held in Baarn, The Netherlands, 14-16 May 1993. Neuromusc. Disord., 4: 75-81.

[68] Tomé, F.M.S., Evangelista, T., Leclerc, A., Sunada, Y., Manole, E., Estournet, B., Barois, A., Campbell, K.P., Fardeau, M. (1994) Congenital

muscular dystrophy with merosin deficiency. C. R. Acad. Sci. Paris, 317: 351-357.

[69] Mercuri, E., Muntoni, F., Berardinelli, A., Pennock, J., Sewry, C., Philpot, J., Dubowitz, V. (1995) Somatosensory and visual evoked potentials in congenital muscular dystrophy: correlation with MRI changes and muscle merosin status. Neuropediatrics, 26: 3-7.

[70] Dubowitz, V., Fardeau, M. (1995) Workshop report: proceedings of the 27th ENMC sponsored workshop on congenital muscular dystrophy. 22-24 april 1994, The Netherlands. Neuromusc. Disord., 5: 253-258.

[71] Banker, B.Q. (1986) Congenital muscular dystrophy. In: A.G. Engel, B.Q. Banker (Eds.), Myology, Vol 2. McGraw-Hill, New York, pp. 1367-1382.

[72] Walton, J.N., Gardner-Medwin, D. (1988) Congenital muscular dystrophy. In: J.N. Walton (Ed.), Disorders of voluntary muscle, 5th ed. Churchill Livingstone, Edinburgh-London-Melbourne-New York, pp. 548-551.

[73] Donner, M., Rapola, J., Somer, H. (1975) Congenital muscular dystrophy: a clinico-pathological and follow-up study of 15 patients. Neuropädiatrie, 6: 239-258.

[74] Trevisan, C.P., Carollo, C., Segalla, P., Angelini, C., Drigo, P., Giordano, R. (1991) Congenital muscular dystrophy: brain alterations in an unselected series of Western patients. J. Neurol. Neurosurg. Psychiatry, 54: 330-334.

[75] Emery, A.E. (1991) Population frequencies of inherited neuromuscular diseases: a world survey. Neuromusc. Disord., 1: 19-29.

[76] Arikawa, E., Ishihara, T., Nonaka, I., Sugita, H., Arahata, K. (1991) Immunocytochemical analysis of dystrophin in congenital muscular dystrophy. J. Neurol. Sci., 105: 79-87.

[77] Morandi, L., Gussoni, E., Mora, M., Gebbia, M., Merlini, L., Cornelio, F. (1990) Dystrophin analysis in congenital muscular dystrophies [abstract]. J. Neurol. Sci., 98(Suppl): 436.

[78] Hayashi, Y.K., Engvall, E., Arikawa-Hirasawa, E., Goto, K., Koga, R., Nonaka, I., Sugita, H., Arahata, K. (1993) Abnormal localization of laminin subunits in muscular dystrophies. J. Neurol. Sci., 119: 53-64.

[79] Kobayashi, O., Yamanouchi, Y., Ozawa, E., Nonaka, I. (1994) Preserved dystrophin-associated glycoproteins in non-Fukuyama type congenital muscular dystrophy [abstract]. Muscle Nerve (Suppl 1): S99.

[80] Hillaire, D., Leclerc, A., Fauré, S., Topaloglu, H., Chiannilkulchaï, N., Guicheney, P., Grinas, L., Legos, P., Philpot, J., Evangelista, T., Routon, M.-C., Mayer, M., Pellissier, J.-F., Estournet, B., Barois, A., Hentati, F.,

Feingold, N., Beckmann, J.S., Dubowitz, V., Tomé, F.M.S., Fardeau, M. (1994) Localization of merosin-negative congenital muscular dystrophy to chromosome 6q2 by homozygosity mapping. Hum. Mol. Genet., 3: 1657-1661.

[81] Kalyanaraman, K., Kalyanaraman, U.P., Lee, R.H. (1980) Autosomal dominant congenital muscular dystrophy (CMD) with possible central nervous system (CNS) involvement [abstract]. J. Neuropathol. Exp. Neurol., 39: 366.

[82] Burgeson, R.E., Chiquet, M., Deutzmann, R., Ekblom, P., Engel, J., Kleinman, H., Martin, G.R., Meneguzzi, G., Paulsson, M., Sanes, J., et al. (1994) A new nomenclature for the laminins. Matrix Biol., 14: 209-211.

[83] Timpl, R., Brown, J.C. (1994) The laminins. Matrix Biol., 14: 275-281.

[84] Mercuri, E., Dubowitz, L., Berardinelli, A., Pennock, J., Jongmans, M., Henderson, S., Muntoni, F., Sewry, C., Philpot, J., Dubowitz, V. (1995) Minor neurological and perceptuo-motor deficits in children with congenital muscular dystrophy: correlation with brain MRI changes. Neuropediatrics, 26: 156-162.

[85] Malik, S., Cruse, R.P., Chou, S.M., Chafel, T. (1990) Non-Fukuyama congenital muscular dystrophy with central nervous system involvement: clinical, radiographic and pathological correlates [abstract]. Ann. Neurol., 28: 430.

[86] Voit, T., Sewry, C.A., Meyer, K., Hermann, R., Straub, V., Muntoni, F., Kahn, T., Unsöld, R., Helliwell, T.R., Appleton, R., Lenard, H.G. (1995) Preserved merosin M-chain (or laminin-α_2) expression in skeletal muscle distinguishes Walker-Warburg syndrome from Fukuyama muscular dystrophy and merosin-deficient congenital muscular dystrophy. Neuropediatrics, 26: 148-155.

[87] Chijiiwa, T., Nishimura, M., Inomata, H., Yamana, T., Narazaki, O., Kurokawa, T. (1983) Ocular manifestations of congenital muscular dystrophy (Fukuyama type). Ann. Ophthalmol., 15: 921-928.

[88] Dobyns, W.B,. Truwit, C.L. (1995) Lissencephaly and other malformations of cortical development: 1995 update. Neuropediatrics, 26: 132-147.

[89] Takeshita, K., Yoshino, K., Kitahara, T., Nakashima, T., Kato, N. (1977) Survey of Duchenne type and congenital type of muscular dystrophy in Shimane. Jinrui-Idengaku-Zasshi, 22: 43-47.

[90] Yoshioka, M,. Sawai, S. (1988) Congenital muscular dystrophy (Fukuyama type): changes in the white matter low density on CT. Brain Dev., 10: 41-44.

[91] Segawa, M., Nomura, Y., Hachimori, K., Shinoyama, N., Hosaka, A., Mizuno, Y. (1979) Fukuyama type congenital muscular dystrophy as a natural model of childhood epilepsy. Brain Dev., 2: 113-119.

[92] Yoshioka, M., Saiwa, S., Kuroki, S., Nigami, H. (1991) MR imaging of the brain in Fukuyama-type congenital dystrophy. A.J.N.R., 12: 63-65.

[93] Nonaka, I., Miyoshino, S., Miike, T., Ueno, T., Usuku, G. (1972) An electron microscopical study of the muscle in congenital muscular dystrophy. Kumamoto Med. J., 25: 68-82.

[94] Murakami, T., Konishi, Y., Takamiya, M., Tsukagoshi, H. (1975) Congenital muscular dystrophy associated with micropolygyria: report of two cases. Acta Pathol. Jpn., 25: 599-612.

[95] Kinoshita, M., Iwasaki, Y., Wada, F., Segawa, M. (1980) A case of congenital olymyositis: a possible pathogenesis of "Fukuyama type congenital muscular dystrophy." Clin. Neurol. (Tokyo), 20: 911-916.

[96] Matsumura, K., Nonaka, I., Campbell, KP. (1993) Abnormal expression of dystrophin-associated proteins in Fukuyama-type congenital muscular dystrophy. Lancet, 341: 521-522.

[97] Nomura, Y., Ehara, M., Yamanaka, T., Arahata, K., Nonaka, I., Segawa, M. (1990) Heterogeneity of congenital muscular dystrophy (CMD) [abstract]. J Neurol Sci 98(Suppl): 437.

[98] Toda, T., Segawa, M., Nomura, Y., Nonaka, I., Masuda, K., Ishihara, T., Suzuki, M., Tomita, I., Origuchi, Y., Ohno, K., Misugi, N., Sasaki, Y., Takada, K., Kawai, M., Otani, K., Murakami, T., Saito, K., Fukuyama, Y., Shimizu, T., Kanazawa, I., Nakamura, Y. (1993) Localization of a gene for Fukuyama type congenital muscular dystrophy to chromosome 9q31-33. Nature Genet., 5: 283-286.

[99] Iida, M., Kurisaki, H., Takatsu, S., Takamiya, M. (1981) A histopathology of spinal anterior horns and roots in congenital and Duchenne types progressive muscular dystrophy. In: Sobue (Ed.), The 1980 annual report of the research group on the clinico-pathology and epidemiology of progressive muscular dystrophy. Ministry of Health and Welfare of Japan, Tokyo, 1981.

[100] Takada, K., Nonaka, I., Shioda, K., Hasebe, T., Takada, C. (1981) Two autopsied cases of Fukuyama type congenital muscular dystrophy, with particular reference to morphometric analysis of the anterior spinal roots [abstract]. Brain Dev., 3: 165.

[101] Takada, K., Nakamura, H., Tanaka, J. (1984) Cortical dysplasia in congenital muscular dystrophy with central nervous system involvement (Fukuyama type). J. Neuropathol. Exp. Neurol., 43: 395-407.

[102] Kamoshita, S., Konishi, Y., Segawa, M., Fukuyama, Y. (1976) Congenital muscular dystrophy as a disease of the central nervous system. Arch. Neurol., 33: 513-516.

[103] Osawa, M., Arai, Y., Ikenaka, H., Murasugi, H., Sugahara, N., Sumida, S., Okada, N., Shishikura, H., Suzuki, H., Hirayama, Y., Hirasawa, K., Fukuyama, Y., Tsutsumi, A., Ito, K., Uchida, Y. (1991) Fukuyama type congenital progressive muscular dystrophy. Acta Paediatr. Jpn., 33: 261-269.

[104] Santavuori, P., Somer, H., Sainio, K., Rapola, J., Kruus, S., Nikitin, T., Ketonen, L., Leisti, J. (1989) Muscle-eye-brain disease (MEB). Brain Dev., 11: 147-153.

[105] Pampiglione, G., Harden, A. (1977) So-called neuronal ceroid lipofuscinosis: neurophysiological studies in 60 children. J. Neurol. Neurosurg. Psychiatry, 40: 323-330.

[106] Santavuori, P., Rapola, J., Sainio, K., Raitta, C. (1984) A variant of Jansky-Bielschowsky disease. Neuropediatrics, 13: 135-141.

[107] Valanne, L., Pihko, H., Katevuo, K., Karttunen, P., Somer, H., Santavuori, P. (1994) MRI of the brain in muscle-eye-brain (MEB) disease. Neuroradiology, 36: 473-476.

[108] Williams, R.S., Swisher, C.N., Jennings, M., Ambler, M., Caviness, V.S. Jr. (1984) Cerebro-ocular dysgenesis (Walker-Warburg syndrome): neuropathologic and etiologic analysis. Neurology, 34: 1531-1541.

[109] Heggie, P., Grossniklaus, H.E., Roessmann, U., Chou, S.M., Cruse, R.P. (1987) Cerebro-ocular dysplasia-muscular dystrophy syndrome: report of two cases. Arch. Ophthalmol., 105: 520-524.

[110] Dambska, M., Wisniewski, K., Sher, J., Solish, G. (1982) Cerebro-oculo-muscular syndrome: a variant of Fukuyama congenital cerebromuscular dystrophy. Clin. Neuropathol., 1: 93-98.

[111] Ranta, S., Pihko, H., Santavuori, P., Tahvanainen, E., De la Chapelle, A. (1995) Muscle-eye-brain disease and Fukuyama type congenital muscular dystrophy are not allelic. Neuromusc. Disord., in press.

[112] Chemke, J., Czernobilsky, B., Mundel, G., Barishak, Y.R. (1975) A familial syndrome of central nervous system and ocular malformations. Clin. Genet., 7: 1-7.

[113] Toda, T., Yoshioka, M., Nakahori, Y., Kanazawa, I., Nakamura, Y., Nakagome, Y. (1995) Genetic identity of Fukuyama-type congenital muscular dystrophy and Walker-Warburg syndrome. Ann. Neurol., 37: 99-101.

[114] Yoshioka, M., Kuroki, S., Nigami, H., Kawai, T., Nakamura, H. (1992) Clinical variation within sibships in Fukuyama-type congenital muscular dystrophy. Brain Dev., 14: 334-337.

[115] Santavuori, P., Pihko, H., Sainio, K., Lappi, M., Somer, H., Haltia, M., Raitta, C., Ketonen, L., Leisti, J. (1990) Muscle-eye-brain disease and Walker-Warburg syndrome [letter]. Am. J. Med. Genet., 36: 371-372.

Chapter III

COMMON TYPES OF CONGENITAL MUSCULAR DYSTROPHY

CLINICAL STUDIES

Congenital muscular dystrophy

Q. H. Leyten, F.J.M. Gabreëls, W.O. Renier,
H.J. ter Laak, R.C.A. Sengers, R.A. Mullaart

The Journal of Pediatrics 1989;115:214-221

Abstract

We report the cases of 18 patients with congenital muscular dystrophy, six of whom also have involvement of the central nervous system, corresponding to the Fukuyama type of congenital muscular dystrophy. In four patients, both the central nervous system and the eyes are involved, and the diagnosis of "muscle, eye and brain disease" was made. A comparative study of these patients with those whose cases were recently reported indicates that there is a wide variability of clinical and laboratory expression of the dystrophy, but a constant feature in all patients is a progression of motor disability. The association of congenital muscular dystrophy with brain abnormalities indicates a poor clinical prognosis. At present it remains an open question whether the three variants of the disease are separate diseases or only different expressions of the same syndrome, but our study tends to support the latter hypothesis.

Congenital muscular dystrophy (CMD) is a rare and ill-defined muscular disorder characterized by generalized weakness and hypotonia at birth, joint contractures, delayed motor development, and features of dystrophy on muscle biopsy. The disorder is slowly progressive, although rapid evolution has been noted occasionally. The clinical severity of CMD and the delay in motor development can differ substantially. Initially the patient's arms are more severely affected than his legs, and proximal muscles more than distal ones. Joint contractures may be restricted to talipes or may constitute a severe form of arthrogryposis multiplex congenita.[1-16] Creatine kinase levels vary and sometimes are only moderately raised at the onset of the disease.[4-16] In general, an autosomal recessive mode of inheritance is accepted,[10,17-19] although some reports describe an autosomal dominant mode of inheritance in "pure" CMD.[20,21]

Apart from the "pure" form of CMD, there are two types of CMD with involvement of the central nervous system: (1) the Fukuyama type[10] and (2) "muscle, eye, and brain disease" (Santavuori et al.[22]), "congenital cerebro-ocular-myopathy syndrome" (Pavone et al.[23]), or "cerebro-ocular dysplasia-muscular dystrophy syndrome" (Towfighi et al.[17]). The neuromorphologic alterations of the CNS are nonspecific and consist of malformations that appear at different stages of brain development, ranging from the fourth week (agenesis of olfactory bulbs) to the final months of gestation (cortical polymicrogyria).[23] The ophthalmologic signs in MEB disease include both functional disturbances, such as nystagmus and uncontrolled eye movements of central origin, and structural eye changes, such as microphthalmia, cleavage defects of the anterior chamber resulting in congenital glaucoma, optic cup deformation resulting in severe myopia, cataract, and retinal changes such as retinal dysplasia and detachment.[17,24]

We report the cases of 18 patients with CMD, of whom six have the Fukuyama type and four the MEB disease type. The purpose of this report is to describe the broad clinical spectrum and to discuss the different nosologic entities.

Methods

In a period of about 15 years we examined and followed 18 patients with CMD from a consecutive series of 1980 patients who had muscle biopsies. All patients with generalized weakness and hypotonia at birth or a severely delayed motor development, or both, underwent muscle biopsy of the quadriceps. Two speci-

mens were taken from the biopsy; the one used for enzyme histochemical procedures was frozen in isopenthane cooled in liquid nitrogen and was cut and stained according to standard procedures, and the other was taken for biochemical measurements. The final diagnosis of CMD was based on histologic signs of muscular dystrophy.

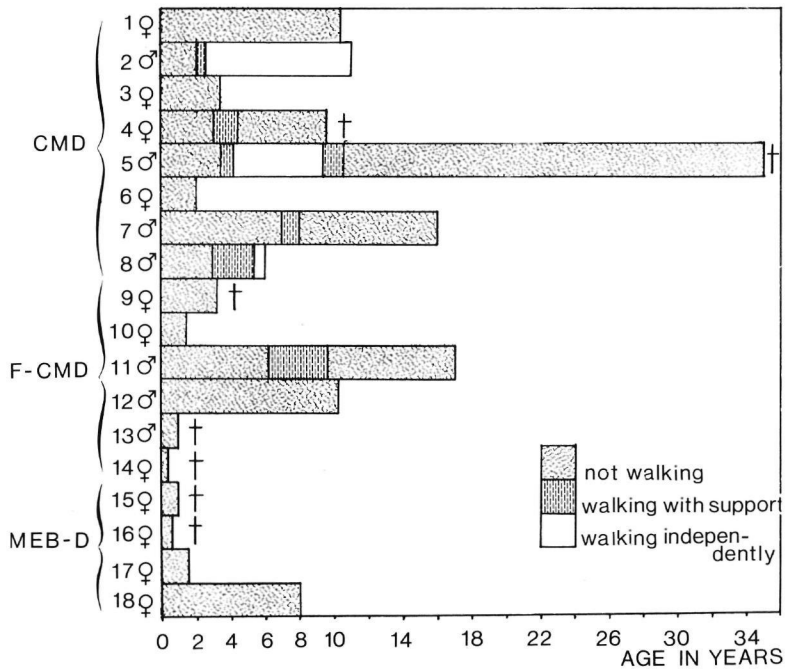

Fig. 1. Motor development of eight patients with CMD, six with Fukuyama-CMD, and four with MEB disease. Age of ambulation at the end of follow-up or at death (†).

Results

Clinical features (Table, Fig. 1)

Decreased antenatal movement was noted in five patients. Three patients were born by breech delivery. Mild postnatal asphyxia occurred in five patients. All patients had hypotonia and generalized weakness from birth. In most patients, proximal muscles were more affected than distal ones and, in general, the arms were more impaired than the legs. Contractures were present at birth in four patients and developed between the ages of 3 and 7 years in seven others,

necessitating tendon-lengthening procedures in two patients. Kyphoscoliosis of the thoracolumbar spine occurred in seven of the patients with "pure" CMD, six of whom also had a congenital subluxation or a dislocation of the hips.

The CNS was involved in 10 patients and the eyes in four of them. Mental retardation occurred in all ten of these patients and varied from mild in two patients with Fukuyama-CMD to severe in the other eight patients. Two patients had tonic-clonic seizures. The four patients with MEB disease had either microcephaly or macrocephaly. Two of the six patients with Fukuyama-CMD had microcephaly. Either encephalocele or meningocele occurred in three patients.

Ocular involvement included microphthalmos, blepharophimosis, corneal clouding, and fetal anterior chamber angle in two patients; corneal clouding, fetal anterior chamber angle, iris atrophy, and cataract in one; and uncontrolled eye movements, horizontal rotatory nystagmus, severe myopia, and macular atrophy in the fourth.

In all patients, motor development was significantly delayed (Fig. 1); the patients with CNS involvement did not reach any motor milestones. In the CMD patients without CNS involvement, three patients were able to walk without support and one walked from the age of 3 to 12 years. Two patients with "pure" CMD died at the ages of 9.5 and 35 years, three patients with Fukuyama-CMD at the ages of 3, 0.8, and 0.3 years, and two patients with MEB disease at the ages of 3 months and 2 days, respectively.

Genetic aspects

The male/female ratio was 7:11. There were four sets of familial cases: two sisters in two families, a father and daughter, and a brother and sister. In only one case consanguinity was proved.

Biochemistry

Results of the following laboratory tests were normal: complete blood cell count and urinalysis, renal function tests, and determinations of serum electrolyte levels, serum protein content and electrophoretic pattern, serum lipid spectrum, serum pH, carbon dioxide tension and bicarbonate value, ceruloplasmin, copper, ammonia, amino acid, and organic acid levels in plasma and urine, and lysosomal enzyme activities in leukocytes. Appropriate studies ruled out endocrinologic, immunologic, and chronic infectious diseases, vitamin deficiencies, and disorders caused by toxic agents.

Table. Signs, symptoms, and laboratory, radiologic, and neurophysiologic characteristics of 18 patients with CMD

	"Pure" CMD patient No							
	1	2	3	4	5	6	7	8
Age at examination (yr)	0 5	3	1	3	35	0 7	6	6
Sex	F	M	F	F	M	F	M	M
Consanguinity	–	–	–	–	–	–	–	–
Delivery, position	N	N	B	B	N	N	N	N
Asphyxia in neonatal period	+	–	–	–	–	–	+	–
Congenital hypotonia	+	+	+	+	+	+	+	+
Joint contractures	+	–	–	+	+	+	+	+
At birth	–	–	–	+	+	–	–	+
At later stages	+	–	–	+	+	+	+	+
Rapidly progressive course	+	–	–	+	–	–	–	–
CK activity (normal <90 U/L)	288	134	N	128	N	N	282	143
Lactate in serum	N	N	N	N	np	N	N	N
Myogenic pattern of EMG	+	+	+	+	+	+	+	+
OFC	N	N	N	N	N	N	N	>P97 5
CNS involvement	–	–	–	–	–	–	–	–
Mental retardation	–	–	–	–	–	–	–	–
Convulsions	–	–	–	–	–	–	–	–
CSF abnormalities	np	np	–	np	np	–	np	np
EEG abnormalities	np	np	–	np	np	np	np	–
Hydrocephalus	–	–	–	–	–	–	–	–
Hypomyelination	–	–	–	–	–	–	–	–
Encephalocele	–	–	–	–	–	–	–	–
Ophthalmologic disorder	–	–	–	–	–	–	–	–

+, Present, –, not present, EMG, electromyography, OFC, occipitofrontal circumference, N, normal, B, breech delivery, np, not performed, EEG, electroencephalography, P, percentile
*Increased protein

Fukuyama-CMD: patient No.						MEB disease: patient No.			
9	10	11	12	13	14	15	16	17	18
1	0.5	9	2	0.3	0	0.3	0.2	0.6	1.6
F	F	M	M	M	F	F	F	F	F
–	–	–	–	–	–	–	–	–	+
B	N	N	N	N	N	N	N	N	N
–	–	–	+	–	–	+	+	–	–
+	+	+	+	+	+	+	+	+	+
+	+	+	+	–	–	–	–	–	+
–	–	+	–	–	–	–	–	–	–
+	+	+	+	–	–	–	–	–	+
+	+	–	–	+	+	+	+	+	–
590	527	N	N	690	80	1540	1130	990	1495
np	N	N	N	np	np	N	N	N	N
+	+	+	+	+	np	+	+	+	+
<P2 5	<P2.5	P90	N	P90	P90	>P97.5	>P97.5	<P2.5	N
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	–	–	–	–	–	–	+	–	–
–	–	np	–	–	np	+*	–	–	–
+	+	+	+	+	+	+	+	+	+
+	+	–	–	+	+	+	+	+	–
–	–	+	–	–	–	–	–	–	+
–	–	–	–	–	–	+	+	+	–
–	–	–	–	–	–	+	+	+	+

Lactate and pyruvate levels in serum and cerebrospinal fluid were determined in 10 patients, and were within normal ranges; 24-hour lactate excretion in urine was normal. Further studies of CSF showed normal leukocyte count, normal protein electrophoresis and immunoelectrophoresis, and normal concentrations of ketone bodies; an elevated CSF protein content (1576 mg/L) was found in only one child with MEB disease.

Five patients had normal CK activity and nine slightly increased CK activity; in the latter, CK activity decreased during progress of the disorder. The activity of CK was markedly increased in the patients with MEB disease.

Neurophysiologic and radiologic examinations (Table)

Electroencephalography showed diffuse slowing in the patients with CNS involvement, and paroxysmal discharges in two patients. The visual evoked potentials and brain-stem auditory evoked potentials were severely disturbed in two patients with CNS involvement. In all patients, electromyographic studies indicated a myopathic disorder by an increased number of brief polyphasic potentials with small amplitude; motor nerve conduction velocities were normal in all patients. In one patient with Fukuyama-CMD, somatosensory evoked potentials could not be elicited with normal sensory action potentials of peripheral nerves, and in another they indicated diffuse involvement of the CNS. On CT scan the third and lateral ventricles were significantly enlarged in all but one patient with combined CNS and ocular involvement. Cerebral and cerebellar atrophy or hypoplasia was observed in five patients. In one patient with the Fukuyama type and one with MEB disease, a hypodense area was present in the left temporooccipital lobe and the frontal lobe, respectively.

Muscle biopsies (Fig. 2)

Light microscopy. In all cases there was an increased variation in fiber diameter caused by the presence of atrophic and hypertrophic fibers. All biopsy specimens were characterized by rounded muscle fibers surrounded by increased quantities of collagen. In all biopsy specimens except one, basophilic fibers were also present. Fatty infiltration was seen in 10 cases and an increased number of fibers with internal nuclei occurred in eight. Necrotic fibers were present in four cases. Small numbers of hyalin fibers were found in 13 cases. The terminal innervation ratio was normal in all patients.

Biochemical studies. Pyruvate oxidation and fatty acid oxidation were in the normal range. Carnitine content was examined in five cases; no abnormalities were found.

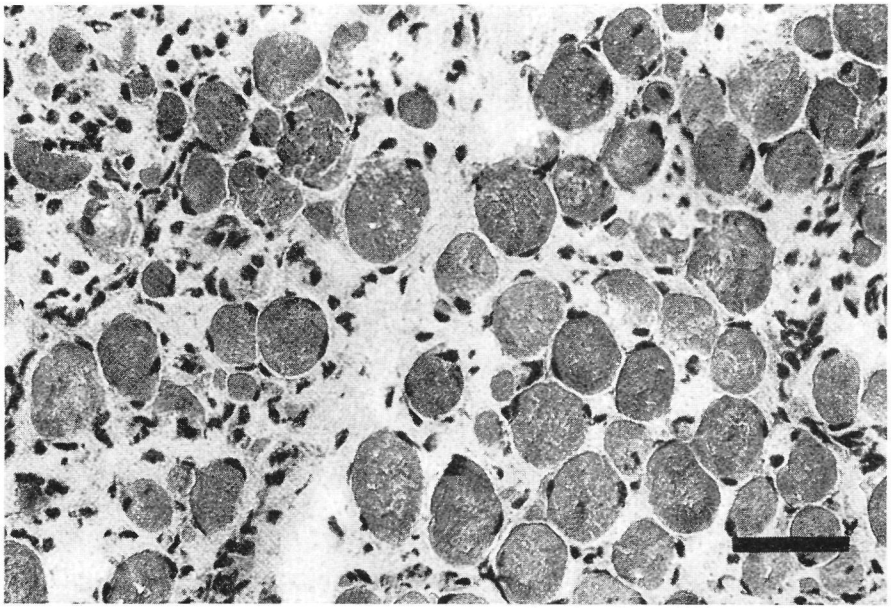

Fig. 2. Transverse section of quadriceps muscle biopsy specimen from patient 1. There is a considerable variation in muscle fiber diameter. Between the rounded muscle fibers, increased quantities of connective tissue can be seen (Hematoxylin-eosin stain; bar, 50 μm).

Pathologic examination

Pathologic findings in two patients with Fukuyama-CMD (Nos. 13 and 14) have been described by Krijgsman et al.[25]

Two patients with MEB disease are still alive; in the other two patients (Nos. 15 and 16, who were sisters) autopsy was performed. Brain examination revealed agyria, polymicrogyria and pachygyria, thickened arachnoidea, hydrocephalus, agenesis of the olfactory bulbs, optic chiasm and septum pellucidum, cerebellar hypoplasia, and an encephalocele above the cerebellum. On light microscopic examination, we observed a stratification disorder, hypomyelination of cerebral white matter, diffuse leptomeningeal and marginal glial proliferation, and vessel hypertrophy. Both eyes had malformations consisting of diffuse disruption of laminar order and dysplastic and dysgenetic changes.

Discussion

According to the criteria of Banker,[13] the clinical and pathologic features of our 18 patients fit the diagnosis of CMD. Ten of these patients also have CNS involvement, and four of them have additional ocular involvement.

Many clinical data in our study are in accordance with those of the literature.[6-9] All patients had generalized hypotonia and generalized muscle weakness at birth, indicating that the disease starts during intrauterine life, but contractures were not always present.[5,8,14,15] A prolonged delivery time[8,10] could not be confirmed; there was a high incidence of breech deliveries, and, in four cases, perinatal cyanosis and drinking or sucking problems were noted.[5,10,12] Progression is rapid and prognosis is worse in MEB disease compared with "pure" CMD.[7,8] In the studies of Donner et al.[8] and McMenamin et al.,[7] 2 of 15 and 8 of 24 patients, respectively, died before the age of 16 years.

In countries other than Japan, Fukuyama-CMD is very rare.[25-33] The CNS manifestations in Fukuyama-CMD include moderate to severe mental retardation, microcephaly, and convulsions. The most common pathologic changes in the CNS are polymicrogyria and agyria (lissencephaly) caused by a defect in the migration of neurons.[10,34] Other neuropathologic changes are hypomyelination of the cerebral white matter,[35] adhesions between the hemispheres, sometimes mild hydrocephalus, and an aberrant pathway of the pyramidal tract. Ophthalmologic involvement does not usually occur.[10,36,37] Among our six patients, one has generalized convulsions. and two, who have microcephaly and severe mental retardation, have enlarged ventricles. Such pronounced hydrocephalus is exceptional in Fukuyama-CMD.[10] One of our patients has a decrease of radiodensity in the white matter, suggesting hypomyelination, as described by Egger el al.,[29] Echenne et al.,[35] and Mukoyama et al.[38]

Santavuori et al.[22] and Raitta et al.[24] first introduced the term "muscle, eye, and brain disease," representing a rare disorder characterized by varying but usually severe mental retardation, macrocephaly, often severe hydrocephalus, hypotonia, and visual failure. Some authors have described encephaloceles.[17,39,40] All but one of our patients had severe hydrocephalus with agyria, and three had encephaloceles.

According to Williams et al.,[41] Towfighi et al.,[17] and Dobyns et al.,[42] many neuropathologic similarities exist in Fukuyama-CMD and MEB disease, although there are some differences; in general, most patients with Fukuyama-CMD have microcephaly with hypomyelination of the cerebral white matter, and they have no or mild dilation of the ventricles, no encephaloceles, and no ocular

involvement, in contrast, patients with MEB disease generally have macrocephaly, and they have hydrocephalus and encephaloceles with severe ophthalmologic involvement Our data support these observations, confirming that on clinical and neuropathologic grounds a distinction between Fukuyama-CMD and MEB disease can be made However, clinical overlap does exist, for instance, two of our patients with typical Fukuyama-CMD had severe hydrocephalus

In 1978, Warburg[43] reported thirteen patients with hydrocephalus, microphthalmos and congenital retinal nonattachment Pagon et al[44] described two siblings with autosomal recessive eye and brain anomalies, of whom one also had an occipital encephalocele, and suggested the term "Warburg syndrome" Initially, muscular dystrophy was not detected in the Warburg syndrome, the muscle disease was probably overshadowed by severe cerebroocular abnormalities Later, however, Towfighi et al[17] and Dobyns et al[42] found typical dystrophic muscular changes, and we share their opinion that there is probably no distinction between the Warburg syndrome and MEB disease

Most of our patients have normal or moderately increased CK activity, as described previously,[7,8,12,21,45] whereas CK values in MEB disease are higher than in other types of CMD In all cases, CK activity decreased with progression of the disorder[1,13] Considering that lactate and pyruvate levels in serum and CSF, and pyruvate oxidation, fatty acid oxidation, and carnitine levels in muscle, are normal in the examined patients, mitochondrial dysfunction is unlikely Mitochondrial abnormalities such as crystalline inclusions and contorted cristae, as found in patient 5,[21] can be interpreted as a secondary phenomenon[46-48] Except for a difference in serum CK activities in "pure" CMD and Fukuyama-CMD on the one hand and in MEB disease on the other, differentiation of the subtypes cannot be made on biochemical grounds

Histologic characteristics of muscle biopsy specimens in "pure" CMD, Fukuyama-CMD, and MEB disease are variable[1,17,40] Dystrophic characteristics generally depend on the stage of the disorder and are usually progressive[8] In our series there was no correlation between age and degree of severity of abnormality Histologic differences between patients with and those without CNS or ophthalmologic involvement could not be found, which is in accordance with the literature[10,17] We found an inflammatory infiltrate in muscle, as described by Towfighi et al[17] in some MEB disease cases, in only one patient with "pure" CMD

The cause of the syndrome of CMD is not known Because of the high incidence of the disorder among siblings, a genetic determination must be considered However, in cerebroocular dysgenesis and in Fukuyama-CMD, some

authors[41,49,50] have suggested a viral meningoencephalitis, presumably of low virulence, as the cause of the proliferative, sclerosing process of the leptomeninges that eventually leads to an internal hydrocephalus. Thickening of the arachnoid with diffuse leptomeningeal and marginal gliodermal proliferation occurred in two of our patients with MEB disease, but antibodies against *Toxoplasma*, cytomegalovirus. herpesvirus, and rubella virus were not increased. Regarding the slight, diffuse mononuclear cell infiltration in patient 1, several authors[17,34,40,51] also observed mild, focal cellular infiltrates in the perimysium and endomysium and around blood vessels. Although the histopathologic findings might suggest a chronic meningoencephalitis active through the second and third trimesters of pregnancy and leading to oculocerebral malformation, the fact that siblings also can be affected indicates a genetic influence. The possibility remains of a nongenetic cause in sporadic cases.

Recently we reported a family (patients 4 and 5) in which the "pure" CMD had an autosomal dominant mode of inheritance.[21] These different modes of inheritance in CMD indicate the likelihood of genetic heterogeneity. Except for reports of this and one other[20] "pure" CMD family with a likely autosomal dominant mode of inheritance, all other reports of "pure" CMD and "CMD plus" in patients mention an autosomal recessive inheritance.[10,17-19,42] Interfamilial differences are more pronounced than intrafamilial ones, which also suggests genetic heterogeneity; the existence of allelic mutations in autosomal recessive CMD, Fukuyama-CMD, and MEB disease is possible.

The question arises whether three different nosologic entities exist in CMD, or whether there is a single entity with variable expression ("pure" CMD, Fukuyama-CMD, and MEB disease).[36] An argument for the latter hypothesis is the fact that the clinical course varies in CMD from mild to severe, the disease severity being generally more pronounced in Fukuyama-CMD and greatest in MEB disease with early death. Because of the higher values of CK activity in these latter patients, disuse of muscles cannot be the cause. Neuropathologic abnormalities are more pronounced in MEB disease than in Fukuyama-CMD, but no truly specific abnormality is found in these syndromes, with the possible exception of encephaloceles in MEB disease. However, the sibling of a patient with encephaloceles in this series had the typical brain and eye malformations of MEB disease but no encephalocele. The apparent intrafamilial concordance for severity and the fact that sibling pairs are always in the same general category indicate true genetic heterogeneity, as is also suggested by the differences in the incidence of Fukuyama-CMD in different gene pools. Whether this etiologic

heterogeneity is caused by allelic mutations or by mutations in different genes remains to be elucidated.

On the basis of this study and the literature, we suggest that patients with CMD can be classified as having "pure" CMD of the autosomal recessive type, "pure" CMD of the autosomal dominant type, Fukuyama-CMD, or MEB disease, as defined by the apparent mode of inheritance and the presence of brain abnormalities with or without ophthalmologic abnormalities. Brain abnormalities in patients with CMD make the prognosis worse.

References

1. Oppenheim H. Über allgemeine und lokalisierte Atonie der Muskulatur (Myotonie) im frühen Kindesalter. Monatsschr Psychiatr Neurol 1900;8:232.
3. Howard R. A case of congenital defect of the muscular system (dystrophia muscularis congenita) and its association with congenital talipes equinovarus. Proc R Soc Med 1908;1:157-66.
3. Ullrich O. Kongenitale, atonisch-sklerotische Muskeldystrophie. Monatsschr Kinderheilkd 1930;47:502-10.
4. Gubbay SS, Walton JN, Pearce GW. Clinical and pathological study of a case of congenital muscular dystrophy. J Neurol Neurosurg Psychiatry 1966; 29:500-8.
5. Vassella F, Mumenthaler M, Rossi E. Congenital muscular dystrophy. In: Walton JN, Canal N, Scarlato G, eds. Muscle disease. Proceedings of an International Congress, Milan, May 19-21, 1969. Excerpta Medica ICS 1970;199:620-3.
6. Fukuyama Y, Kawazura M, Haruna H. A peculiar form of congenital progressive muscular dystrophy: report of fifteen cases. Pediatr Univ Tokyo (Tokyo) 1960;4:5-8.
7. McMenamin JB, Becker LE, Murphy EG. Congenital muscular dystrophy: a clinicopathologic report of 24 cases. J Pediatr 1982;100:692-7.
8. Donner M, Rapola J, Somer H. Congenital muscular dystrophy: a clinicopathological and follow-up study of 15 patients. Neuropädiatrie 1975;6: 239-58.
9. Lazaro RP, Fenichel GM, Kilroy AW. Congenital muscular dystrophy: case and reappraisal. Muscle Nerve 1979;2:349-55.
10. Fukuyama Y, Osawa M, Suzuki H. Congenital progressive muscular dystrophy of the Fukuyama type: clinical, genetic and pathological considerations. Brain Dev 1981;3:1-29.
11. Serratrice G, Cros D, Pellissier J-F, Gastaut J-L, Pouget J. Dystrophie musculaire congénitale. Rev Neurol (Paris) 1980;136:445-72.
12. Rotthauwe HW, Kowalewski S, Mumenthaler M. Kongenitale Muskeldystrophie. Z Kinderheilkd 1969;106:131-62.
13. Banker BQ. Congenital muscular dystrophy. In: Engel AG, Banker BQ, eds. Myology; vol 2. New York: McGraw-Hill, 1986:1367-82.
14. Zellweger H, Afifi A, McCormick WF, Mergner W. Benign congenital muscular dystrophy: a special form of congenital hypotonia. Clin Pediatr (Phila) 1967:6:655-63.

15. Zellweger H, Afifi A, McCormick WF, Mergner W. Severe congenital muscular dystrophy. Am J Dis Child 1967;114:591-602.
16. Fenichel GM. Bazelon M. Myopathies in search of a name: benign congenital forms. Dev Med Child Neurol 1966:8:532-48.
17. Towfighi J, Sassani JW, Suzuki K, Ladda RL. Cerebro-ocular dysplasia-muscular dystrophy (COD-MD) syndrome. Acta Neuropathol (Berl) 1984: 65:110-23.
18. McKusick VA. Mendelian inheritance in man. Baltimore: Johns Hopkins University Press, 1983:846-7.
19. Fukuyama Y, Ohsawa M. A genetic study of the Fukuyama type congenital muscular dystrophy. Brain Dev 1984;6:373-90.
20. Kalyanaraman K, Kalyanaraman UP, Lee RH. Autosomal dominant congenital muscular dystrophy (CMD) with possible central nervous system (CNS) involvement [Abstract]. J Neuropathol Exp Neurol 1980;39:366.
21. Leyten QH, Gabreëls FJM, Joosten EMG, et al. An autosomal dominant type of congenital muscular dystrophy. Brain Dev 1986;8:533-7.
22. Santavuori P, Leisti J, Kruus S. Muscle, eye and brain disease: a new syndrome [Abstract]. Neuropädiatrie 1977;8(suppl):553.
23. Pavone L, Gullotta F, Grasso S, Vannucchi C. Hydrocephalus, lissencephaly, ocular abnormalities and congenital muscular dystrophy: a Warburg syndrome variant? Neuropediatrics 1986;17:206-11.
24. Raitta C, Lamminen M, Santavuori P, Leisti J. Ophthalmological findings in a new syndrome with muscle, eye and brain involvement. Acta Ophthalmol (Copenh) 1978;56:465-72.
25. Krijgsman JB, Barth PG, Stam FC, Slooff JL, Jaspar HHJ. Congenital muscular dystrophy and cerebral dysgenesis in a Dutch family. Neuropädiatrie 1980;11:108-20.
26. Nonaka I, Chou SM. Congenital muscular dystrophy. In: Vinken PJ, Bruyn GW, eds. Handbook of clinical neurology; vol 41. Amsterdam: Elsevier North-Holland, 1979:27-50.
27. Fowler M, Manson JI. Congenital muscular dystrophy with malformation of the central nervous system. In: Kakulas BA, ed. Clinical studies in myology. New York: Elsevier North-Holland, 1973:192-7.
28. Warter JM, Marescaux C, Coquillat G, Walter P, Micheletti G, Rohmer F. Syndrome autosomique récessif associant myopathie et atteinte du système nerveux central et périphérique. J Neurol Sci 1981;49:135-51.

29. Egger J, Kendall BE, Erdohazi M, Lake BD, Wilson J, Brett EM. Involvement of the central nervous system in congenital muscular dystrophies. Dev Med Child Neurol 1983;25:32-42.
30. Echenne B, Pages M, Marty-Double C, Pages AM, Astruc J, Brunel D. Progressive muscular dystrophy with central nervous system involvement: an original variant of congenital neuromuscular disease [Abstract]. Proceedings of the 5th International Congress on Neuromuscular Diseases, Marseille, France. 1982.
31. Balestrini MR, Mora M, Daniel S. Congenital muscular dystrophy with central nervous system involvement (Fukuyama disease) [Abstract]. Acta Neurol (Napoli) 1982:37:256.
32. Martinelli P, Gabellini AS, Ciucci G, Govoni E, Vitali S, Gulli MR. Congenital muscular dystrophy with central nervous system involvement: case report. Eur Neurol 1987;26:17-22.
33. Korinthenberg R, Palm D, Schlake W, Klein J. Congenital muscular dystrophy, brain malformation and ocular problems (muscle, eye and brain disease) in two German families. Eur J Pediatr 1984;142:64-8.
34. Murakami T, Konishi Y, Takamiya M, Tsukagoshi H. Congenital muscular dystrophy associated with micropolygyria: report of two cases. Acta Pathol Jpn 1975;25:599-612.
35. Echenne B, Arthuis M, Billard C, et al. Congenital muscular dystrophy and cerebral CT scan anomalies: results of a collaborative study of the Société de Neurologie Infantile. J Neurol Sci 1986;75:7-22.
36. Heyer R, Ehrich J, Goebel HH, Christan HJ, Hanefeld F. Congenital muscular dystrophy with cerebral and ocular malformations (cerebro-oculomuscular syndrome). Brain Dev 1986;8:614-8.
37. Miller NR. Muscular dystrophies. In: Miller NR, ed. Walsh and Hoyt's clinical neuro-ophthalmology; vol 2. 4th ed. Baltimore: Williams & Wilkins, 1985:794-811.
38. Mukoyama M, Sobue I, Kumagai T, Negoro T, Iwase K. Fukuyama type congenital muscular dystrophy: autopsied brain and computed tomography. In: Okinaka S, ed. Current research in muscular dystrophy, Japan. Proceedings of the Annual Meeting of the Muscular Dystrophy Research Group. Tokyo, 1977, 1978:102-3.
39. Pagon RA, Chandler JW, Collie WR, et al. Hydrocephalus. agyria, retinal dysplasia, encephalocele (HARD ± E) syndrome: an autosomal recessive condition. Birth Defects 1978;14:233-41.

40. Miyake S, Gota A,. Tsuchida M, Misugi N, Komiya K. An autopsy case of congenital muscular dystrophy associated with hydrocephalus and occipital dermal sinus. Brain Dev 1977;9:212-9.
41. Williams RS, Swisher CN, Jennings M, Ambler M, Caviness VS Jr. Cerebroocular dysgenesis (Walker-Warburg syndrome): neuropathologic and etiologic analysis. Neurology 1984;34:1531-41.
42. Dobyns WB, Kirkpatrick JB, Hittner HM, Roberts RM, Kretzer FL. Syndromes with lissencephaly. II. Walker-Warburg and cerebro-oculo-muscular syndromes and a new syndrome with type II lissencephaly. Am J Med Genet 1985;22:157-95.
43. Warburg M. Hydrocephaly, congenital retinal nonattachment, and congenital falciform fold. Am J Ophthalmol 1978;85:88-94.
44. Pagon RA, Clarren SK, Milam DF Jr, Hendrickson AE. Autosomal recessive eye and brain anomalies: Warburg syndrome. J Pediatr 1983;102: 542-46.
45. Misugi N. Light and electron microscopic studies of congenital muscular dystrophy. Brain Dev 1980;2:191-9.
46. Ito K. Electron microscopic study of human dystrophic muscle. Clin Neurol (Tokyo) 1968;8:685-92.
47. Nonaka I, Miyoshino S, Miike T. Ueno T, Usuku G. An electron microscopical study of the muscle in congenital muscular dystrophy. Kumamoto Med J 1972;25:68-82.
48. Tanimura R. Ultrastructural study of skeletal muscle in floppy infant syndrome: with special emphasis on the cases of central nervous system disorders with marked hypotonia. Acta Paediatr Jpn (Tokyo) 1974;78:470-80.
49. Chan CC, Egbert PR, Herrick MK, Urich H. Oculocerebral malformations: a reappraisal of Walker's "lissencephaly." Arch Neurol 1980;37:104-8.
50. Dambska M, Wisniewski K, Sher J. Solish G. Cerebro-oculo-muscular syndrome: a variant of Fukuyama congenital cerebromuscular dystrophy. Clin Neuropathol 1982;1:93-8.
51. Kinoshita M, Iwasaki Y, Wada F, Segawa M. A case of congenital polymyositis: a possible pathogenesis of "Fukuyama type congenital muscular dystrophy." Clin Neurol (Tokyo) 1980;20:911-6.

White matter abnormalities in congenital muscular dystrophy

Q.H. Leyten, F.J.M. Gabreëls, W.O. Renier, B.G.M. van Engelen,
H.J. ter Laak, R.C.A. Sengers, H.O.M. Thijssen

Journal of the Neurological Sciences 1995;129:162-169

Abstract

Central nervous system (CNS) characteristics were examined in seventeen patients with autosomal recessive classic or "pure" congenital muscular dystrophy (CMD). In three patients, neuroradiological examination (CT/MRI) indicated hypodense white matter areas. Two out of these three patients had epilepsy (seizures and epileptic discharges on their EEG). Only two of the remaining patients had epileptic EEG discharges, but without clinical seizures. By comparing our results to data in the literature, we could conclude that the classic or "pure" form of CMD can be subdivided into two subtypes, i.e. those with and those without white matter hypodensities. A mild form of epilepsy or an epileptic predisposition on EEG can be part of the subtype with white matter hypodensities.

Congenital muscular dystrophy (CMD) has been used to describe a group of neuromuscular disorders characterized by autosomal recessive inheritance, onset at birth or during the first year of life, hypotonia and generalized muscular weakness, multiple joint contractures evident in the first year of life, non-progressive or slowly progressive clinical course, (sub)normal mental development, and muscle biopsies which show striking pathological changes similar to muscular dystrophy and a normal distribution of dystrophin (Fukuyama et al., 1960, 1981; Gubbay et al., 1966; Vassella et al., 1970; Donner et al., 1975; Lazaro et al., 1979; Serratrice et al., 1980; McMenamin et al., 1982; Banker, 1986; Dubowitz, 1994). CMD has been classified into three separate entities (Dubowitz, 1994): classic or "pure" CMD with (sub)normal intelligence, Fukuyama type of CMD (F-CMD) with cerebral abnormalities and severe mental retardation (Fukuyama et al., 1960, 1981), and "muscle-eye-brain disease" (MEB-D) (Raitta et al., 1978; Santavuori et al., 1989; Leyten et al., 1991, 1992) or the "cerebro-ocular dysplasia-muscular dystrophy syndrome" (COD-MD) (Towfighi et al., 1984; Heggie et al., 1987; Federico et al., 1988) with severe mental retardation and ocular malformations. The Walker-Warburg syndrome (WWS) is an autosomal recessive disorder characterized by type II lissencephaly, cerebellar malformation, characteristic eye malformations and congenital muscular dystrophy (McKusick, 1988). At present, there is still no consensus about whether MEB-D and WWS are separate entities (Dubowitz, 1994). Some authors consider that MEB-D and WWS only differ in severity rather than in any fundamental feature (Dobyns et al., 1985; Leyten et al., 1989).

Recently, there have been 14 reports on 64 patients with a "pure" CMD and (sub)normal intelligence, whose CT/MRI scans indicated marked hypodensity of the white matter (Bernier et al., 1979; Nogen, 1980; Egger et al., 1983; Echenne et al., 1986; Martinelli et al., 1987; Yoshioka et al., 1987; Castro-Gago and Peña-Guitián, 1988; Streib and Lucking, 1989; Tanaka et al., 1990; Cook et al., 1992; Kaciński et al., 1992; Pihko et al., 1992; Donner [see Dubowitz, 1994]; Topaloğlu et al., 1994). Topaloğlu et al. (1994) suggested that this type should be considered as an intermediate form between the "pure" form of CMD and the Fukuyama type of CMD, referred to by them as "occidental type cerebro-muscular dystrophy". At present, cases with a (sub)normal intelligence quotient (IQ) who show white matter hypodensity on CT or MRI examination are included into the "pure" form of CMD (Dubowitz, 1994).

We analyzed the CT/MRI of our 17 patients with "pure" CMD and found 3 cases with white matter hypodensities. The clinical, electrophysiological and

neuroradiological data of these patients were compared to those reported in the literature.

Materials and Methods

In a retrospective study on a consecutive series of 2010 patients who underwent muscle biopsy because of muscle weakness, we found 17 patients with CMD. The final diagnosis was based on diagnostic criteria for classic or "pure" CMD (see Table 1) (Dubowitz, 1994). CNS characteristics of these patients were analyzed, including the clinical signs and symptoms, IQ, cerebrospinal fluid (CSF) (9 patients), especially myelin basic protein (MBP), EEG, and neuroradiological findings (CT/MRI). DNA analysis including examination of the Xp21 dystrophy gen was performed on all of the boys with a serum creatine kinase (CK) level of above 150 U/l (normal, <100 U/l), on some of the boys with a normal CK level and on two of the girls.

Reports of 3 CMD cases with white matter hypodensities

Case 15

This 21-year-old man was the only child of healthy non-consanguineous parents. Family history did not mention neuromuscular disorders. Pregnancy was uneventful with normal intrauterine movements. Birth weight was 2870 g. At birth, generalized weakness and hypotonia, facies myopathica and contractures of the hips were found. Respiration was poor during the neonatal period. Motor development was delayed; the patient sat without support at 36 months and pulled himself upright at 40 months. He did not learn to stand and walk without support. Intellectual development was normal.

On admission at 3 years of age, muscle weakness and hypotonia were severe and diffuse, but muscle wasting was not obvious. His face was without expression. Flexion contractures of the elbow, knee, hip and ankle joints were present. Right concave scoliosis was found. The only abnormal neurological signs comprised absent tendon reflexes, while the plantar responses were flexor. There were no dysmorphic features and the head circumference was on the 50th percentile. His mental development was considered to be above average until the age of 3 years.

Table 1. Diagnostic criteria for classic or "pure" CMD

Inclusion criteria	Exclusion criteria
Clinical features:	Clinical features:
– onset at birth or during the first year of life,	– onset during childhood,
– hypotonia and generalized muscular weakness,	– clinical course rapidly progressive,
– multiple joint contractures evident in the first year of life,	– muscular hypertrophy,
– clinical course non-progressive or slowly progressive,	– ptosis and/or ophthalmoplegia,
– normal or subnormal mental development.	– severe impairment of intellectual development (IQ <50),
	– structural ocular abnormalities.
Muscle biopsy:	Laboratory findings:
– dystrophic pattern	– EMG with "neuropathic" pattern,
– marked increase in interstitial connective tissue with or without increase in interstitial adipose tissue,	– muscle biopsy normal, with neuropathic abnormalities or with structural abnormalities specific of other myopathies,
– no marked fibre necrosis and regenerative activity,	– dystrophin absent or abnormal,
– sometimes more pronounced by dystrophic pattern, with evident necrosis and regeneration; dystrophin (both by immunocytochemical and Western-blot techniques) must be present and normal.	– major CT or MRI abnormalities of CNS different from white matter hypodensity (major malformations, developmental and/or migration defects).

On examination at the age of 13 years, there was no evidence of progression of the muscle weakness. The Wechsler Intelligence Scale for Children-Revised (WISC-R) revealed a full scale IQ score of 115.

At the age of 20 years, muscle weakness was static. He did not show deterioration in intelligence and behaviour. At this age, he had suffered two generalized tonic-clonic grand mal epileptic seizures. Over the last four years he developed progressive thoracic scoliosis.

Biochemical investigations of serum and urine gave normal values except for a serum CK of 400 U/l (normal, <100 U/l). Full blood count, electrolytes, lactate and pyruvate levels in serum and CSF, blood and urine amino acids, copper, long chain fatty acids, white cell lysosomal enzymes and organic acids were normal. Microbiological examination excluded infections such as toxoplasma, cytomegalovirus, herpes or rubella virus. Chromosome analysis revealed a normal 46,XY karyotype. Rearrangements of the dystrophy gen at Xp21 were not found.

Electromyography (EMG) revealed an increased number of brief polyphasic potentials with a small amplitude. Motor nerve conduction velocities were normal. EEG showed rhythmic activity which was poor for his age and multifocal discharges. VEP and SSEP were normal.

A biopsy from the quadriceps muscle at the age of 3 years showed a wide variation in fibre diameters; many of the fibres were larger than normal. Degenerative changes were present with central nuclei and fibre splitting. There was an increase in the interstitial connective tissue and some fat replacement.

Cerebral CT at the age of 13 years showed marked hypodensity of the white matter of both hemispheres, which could also be seen on MRI. There has been no progression in white matter hypodensities on CT/MRI during the past 5 years.

Case 16

A boy, the third child of healthy consanguineous parents (second cousins), was born after an uneventful pregnancy and normal intrauterine movements. Family history did not mention any neuromuscular disorders. Birth weight was 3925 g. Postpartum period was not complicated by asphyxia. At birth, generalized weakness and hypotonia, facies myopathica and contractures were found. Since the age of 2 years, he has been receiving (successful) treatment with antiepileptic drugs for partial complex epileptic seizures, sometimes with secondary generalization.

On examination at the age of 6 years, his head circumference was 55 cm (>P90) and strabismus divergens was noted, without any structural ocular malformations. Generalized hypotonia and muscle weakness, which affected the proximal and distal muscles equally, were present with "facies myopathica". Deep tendon reflexes could not be elicited. Examination of the feet revealed "pedes equinovarus adducti" with toe-walking. Further development was characterized by motor retardation and contractures of the achilles tendons which necessitated tendon lengthening procedures. At the age of 7 years, he became wheelchair-dependent. Over the past 8 years he has developed progressive thoracolumbar scoliosis which required spondylodesis. At present he is 22 years old and wheelchair-dependent. He did not show any deterioration in intelligence and behaviour.

Fig. 1. Cerebral CT scan of case 16 showing diffuse hypodensity of the white matter.

Biochemical investigations of serum and urine gave normal values (CK, 67 U/l). Full blood count, electrolytes, lactate and pyruvate levels in serum and CSF, blood and urine amino acids, copper, long chain fatty acids, white cell lysosomal enzymes and organic acids were normal. Microbiological examination excluded infections such as toxoplasma, cytomegalovirus, herpes or rubella virus. Chromosome analysis revealed a normal 46,XY karyotype. Rearrangements of the dystrophy gen at Xp21 were not found.

EMG revealed an increased number of brief polyphasic potentials with a small amplitude. Motor nerve conduction velocities were normal. EEG showed diffuse slowing of the background and paroxysmal epileptic discharges.

A biopsy from the quadriceps muscle at the age of six years revealed dystrophic changes, such as increased variability of muscle fibre size, severe increase of fat cells, basophilic fibres and fibres with internal nuclei and an increased amount of interstitial connective tissue.

Cerebral CT at the age of 12 years showed diffuse hypodensity of the white matter (Fig. 1). MRI of the brain was not performed. No progression of the white matter hypodensities has been seen on CT during the past 3 years.

Case 17

A girl, the third child of healthy non-consanguineous parents was born after an uneventful pregnancy with normal intrauterine movements. Family history did not mention any neuromuscular disorders. Birth weight was 3360 grams and the Apgar scores were 9 and 10. Postpartum period was not complicated by asphyxia. At birth, generalized weakness and hypotonia, facies myopathica and slight dysplasia of the left hip were found. Deep tendon reflexes were absent. Head circumference was normal for her age. Ophthalmological examination did not reveal any structural malformations. At the age of 3 months, torticollis was noted, which necessitated operative correction at the age of 7 months. By the age of 18 months she was able to crawl, but could not stand. Generalized areflexia, hypotonia and muscle weakness were obvious, especially in the proximal musculature. Contractures did not occur. Mental development was normal. The following examinations were performed at this age of 18 months.

Biochemical investigations of serum and urine gave normal values, except for increased CK (1178 U/l; normal, <90). Full blood count, electrolytes, lactate and pyruvate levels in serum and CSF, blood and urine amino acids, copper, long chain fatty acids, white cell lysosomal enzymes and organic acids were normal. Microbiological examination excluded infections such as toxoplasma, cytomegalovirus, herpes or rubella virus. Chromosome analysis revealed a normal 46,XX

karyotype. Rearrangements of the dystrophy gen at Xp21 were not found.

EMG revealed an increased number of brief polyphasic potentials with a small amplitude. Nerve conduction velocity was normal. EEG, VEP and BAEP were normal.

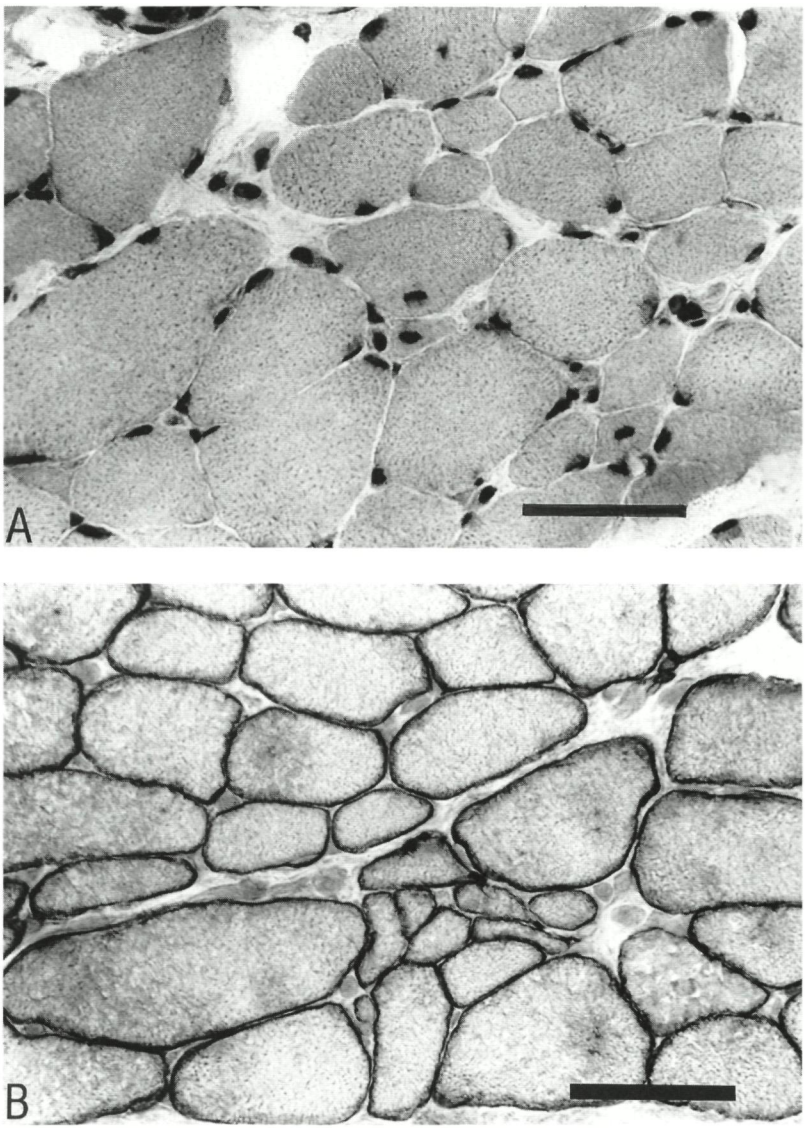

Fig. 2. Biopsy of quadriceps muscle stained with HE (A) and dystrophin antibody (B). Note the wide variation of muscle fibre diameters and the even distribution of dystrophin. Bar = 50 μm.

Table 2. Signs and symptoms, laboratory, genetical, radiological, and neurophysiological characteristics of 17 patients with "pure" congenital muscular dystrophy, 3 of whom had white matter hypodensities

	"Pure" CMD						
	Without white matter hypodensity						
Case No	1	2	3	4	5	6	7
Age at muscle biopsy (yr)	0	0	1	1	1	2	3
Sex	F	M	F	F	F	M	M
Consanguinity	–	–	–	–	+	–	–
Congenital hypotonia	+	+	+	+	+	+	+
Joint contractures	+	–	–	+	+	–	–
Rapidly progressive course (motor retardation)	+	–	–	–	–	+	–
CK activity (normal <90 U/l)	288	N	N	N	N	N	N
DNA analysis	np	np	np	N	np	np	N
Dystrophic muscular biopsy	+	+	+	+	+	+	+
CNS involvement							
– CSF abnormalities	np	np	N	np	N	N	N
– EEG abnormalities	–	–	–	np	–	–	–
– Convulsions / epilepsy	–	–	–	–	–	–	–
– White matter hypodensity (CT/MRI)	np	–	–	–	–	–	–
– Intelligence *	N	N	N	N	N	N	N

N = normal, np = not performed, – = absent, + = present * Normal intelligence = IQ >70

							With white matter hypodensity		
8	9	10	11	12	13	14	15	16	17
3	3	3	4	5	6	14	3	6	1
M	M	M	M	M	M	M	M	M	F
–	–	–	–	–	–	–	–	+	–
+	+	+	+	+	+	+	+	+	+
+	–	–	–	–	–	+	+	+	–
–	–	–	–	+	–	–	+	–	–
143	N	N	134	153	392	N	400	N	1178
np	np	N	np	N	N	np	N	N	N
+	+	+	+	+	+	+	+	+	+
np	N	N	np	np	N	np	np	N	N
+	+	–	–	–	–	–	+	+	–
–	–	–	–	–	–	–	+	+	–
–	–	–	–	–	–	–	+	+	+
N	N	N	N	N	N	N	N	N	N

A biopsy from the quadriceps muscle at the age of 18 months revealed dystrophic changes, such as rounded fibres, a wide variation in the fibre diameter and an increased amount of interstitial connective tissue (Fig 2A) There was no marked evidence of fibre necrosis and regenerative activity Immunohistochemistry with dystrophin antibodies showed a normal expression pattern (Fig 2B)

Cerebral CT at the age of 18 months showed hypodensities in the white matter At this age, MRI revealed hypodensities in the periventricular areas of both occipital horns (T1, sagittal) and hyperintensity of the white matter (T2) No progression in white matter hypodensities has been seen on CT/MRI during the past 3 years

Clinical course was characterized by motor retardation, but with normal mental development

Analysis of the data on 17 patients with CMD (Table 2)

Neuroradiological findings

Thirteen patients did not show abnormalities on their CT or MRI of the brain In the 3 patients (cases 15, 16 and 17) with white matter hypodensity on cerebral CT, the hypodensity was diffuse and could be confirmed in 2 of the 3 cases by MRI, in one patient, MRI was not available (case 16) The first neuroimaging occurred at the age of 1 5, 13 and 16 years, respectively Follow-up was performed 3, 5 and 3 years later, respectively There was no change or progression

Clinical features

There were no clinical differences between CMD with and without white matter abnormalities

EEG and epilepsy

In cases 8 and 9, generalized spike-wave paroxysms occurred during sleep In case 8, there were also some spikes of short duration in the central regions, while in case 9, paroxysmal sharp theta waves were found, but these electrical phenomena were not associated with seizures

Two patients with white matter hypodensities (cases 16 and 17) had poor rhythmic background activity for their age In cases 16 and 17, the following were noted multifocal discharges, diffuse slowing of the background activity

and paroxysmal epileptic discharges, such as bilateral irregular sharp theta waves which were sometimes mingled with spikes. Both patients had clinical seizures. Case 16 had suffered from two generalized tonic-clonic epileptic seizures at the age of 20 years, while case 17 had suffered partial complex seizures sometimes with secondary generalization from the age of 2 years onwards. Seizures could be treated successfully using conventional antiepileptic drugs.

Laboratory findings

In 16 out of our 17 patients, serum CK activity appeared to be normal or slightly increased. One case (case 17) with cerebral white matter hypodensity had markedly increased CK activity.

All the CSF parameters, including cell count, protein content, protein electrophoresis, immuno-electrophoresis, myelin basic protein, lactate and pyruvate levels, were within normal ranges.

Genetics

The male/female ratio was 12:5. In 2 patients, consanguinity could be proved (case 5, 8^{th}-9^{th} generation; case 16, second cousins).

DNA analysis was performed on 6 boys and 2 girls. Rearrangements of the dystrophy gen at Xp21 were not found.

Discussion

Congenital muscular dystrophy may be associated with more or less marked disorders of cerebral development. There are three subtypes (Dubowitz, 1994): classic or "pure" CMD, F-CMD and MEB-D. Topaloğlu et al. (1994) believe that CMD with only white matter hypodensities on CT or MRI examination should be considered as an intermediate form. However, according to the opinion of most of the participants in a workshop on this topic (Dubowitz, 1994), cases with a (sub)normal IQ who show white matter hypodensities on CT or MRI, without major malformations, developmental and/or migration defects of CNS, should be included in the classic or "pure" form of CMD (see Table 1).

Table 2 shows a summary of the clinical data published in the literature on patients with CMD, (sub)normal intelligence, and low density areas in the white matter. All 17 patients with CMD in our series showed normal intellectual development. Three had marked, diffuse hypodensity of the white matter on CT and/or MRI examination. There were no dysmorphic features and no pyramidal signs. Some reports mentioned dysmorphic features, such as a long and thin

Table 3. Review of previously reported cases (64) and our cases (3) of congenital muscular dystrophy with (sub)normal intelligence and low density areas in the white matter

	(First) author (year of publication)						
	Bernier (1979)	Nogen (1980)	Egger (1983)	Echenne (1986)	Martinelli (1987)	Yoshioka (1987)	Castro-Gago (1988)
Number of cases	5	2	3	6	1	1	1
Male : female ratio	3 : 2	0 : 2	1 : 2	4 : 2	1 : 0	1 : 0	1 : 0
Weakness							
– nonprogressive	5/5	2/2	2/3	4/6	0/1	1/1	1/1
– progressive	0/5	0/2	1/3	2/6	1/1	0/1	0/1
Epilepsy	1/5	0/2	1/3	2/6	1/1	0/1	0/1
IQ > 70	5/5	0/2	3/3	1/6	1/1	1/1	1/1
50-70	0/5	2/2	0/3	5/6	0/1	0/1	0/1
Other clinical signs							
– dysmorphic features *	0/5	0/2	0/3	0/6	0/1	1/1	0/1
– pyramidal syndrome	0/5	0/2	0/3	0/6	0/1	0/1	0/1
Epileptic EEG	1/5	0/2	1/3	2/4	1/1	0/1	0/1

F = female; M = male; nm = not mentioned, * Dysmorphic features, such as (long and) thin face, high arched palate, abnormalities of jaw articulation, and pigeon chest.

Table 3. (continued)

	(First) author (year of publication)								
	Streib (1989)	Tanaka (1990)	Kacıński (1992)	Cook (1992)	Pihko (1992)	Donner **	Topaloğlu (1994)	Our cases	Total
Number of cases	2	1	1	11	5	5	20	3	67
Male female ratio	1 1	1 0	0 1	4 7	3 2	nm	12 8	2 1	34 28
Weakness									
– nonprogressive	2/2	1/1	1/1	2/11	5/5	nm	3/20	2/3	31/62
– progressive	0/2	0/1	0/1	9/11	0/5	nm	17/20	1/3	31/62
Epilepsy	2/2	0/1	0/1	0/11	2/5	3/5	1/20	2/3	15/67
IQ > 70	2/2	1/1	1/1	9/11	5/5	5/5	11/13	3/3	49/60
50-70	0/2	0/1	0/1	2/11	0/5	0/5	2/13	0/3	11/60
Other clinical signs									
– dysmorphic features *	0/2	0/1	0/1	0/11	0/5	nm	8/20?	0/3	9/62
– pyramidal syndrome	1/2	0/1	0/1	0/11	0/5	nm	0/20	0/3	1/62
Epileptic EEG	2/2	0/1	0/1	1/4	5/5	nm	8/20?	2/3	25/53

** See Dubowitz 1994

face, abnormalities of jaw articulation and a high arched palate (Yoshioka et al., 1987; Topaloğlu et al., 1994). Although the authors of these reports suggested that these clinical aspects are specific, we believe that the facial dysmorphic features and high arched palate are not specific for this group, but they are frequently seen in myopathic children (Leyten et al., 1990). Topaloğlu et al. (1994) reported clinical findings in 18 cases with "pure" CMD and 20 cases with (sub)normal IQ which show white matter hypodensity. The last group may tend to run a more severe course in the presence of significantly higher CK levels. This is in agreement with our findings.

EEG abnormalities are not uncommon in "pure" CMD. Spikes and slow waves, periodic complexes and focal spikes can be detected in the routine EEG recordings (Pihko et al., 1992, Topaloğlu et al., 1994). In literature 21 of 50 studies cases with hypodensity of the white matter had abnormal recordings (see Table 3). Two of our three patients (cases 15 and 16) had seizures and epileptic discharges on their EEG. In contrast, only 2 of the 14 remaining CMD cases (cases 8 and 9) had epileptic EEG discharges without any seizures. However, only CT but no MRI examination of the brain had been performed on these patients, so we cannot exclude minor CNS deficit which can only be visualized using MRI.

The occurrence of epilepsy was mentioned in 13 out of 64 patients in the literature (Table 3). Based on the data presented in Table 3, it can be concluded that there is a significantly higher epilepsy ratio (1 : 4.5) in CMD patients with (sub)normal intelligence and associated white matter hypodensities than in the normal population (1 : 150). This phenomenon cannot be explained by other neurological pathology. Therefore we believe that a mild form of epilepsy can be part of CMD with white matter hypodensities.

The nature and significance of the white matter changes remain obscure. Informative neuropathological data are scarce. There is only one report (Egger et al., 1983) which described two patients. One patient showed moderate focal subpial gliosis and good preservation of the cortical architecture in a brain biopsy from the right frontal lobe. The myelin sheaths were intact in the core of the convolution. In the white matter, there was obvious astrocytic proliferation. This patient had a WISC-IQ of 135 at the age of 8 years, but this had fallen to 90 at the age of 10 years. In the other patient in the report, microscopical examination showed patchy demyelination of the white matter of the centrum semiovale. The authors suggest that these neuropathological findings in themselves were not diagnostic, but the low density in the central areas of the brain on the CT scans may indicate a demyelinating process. One other case had

spongy appearance of white matter on necropsy (Echenne et al., 1986). Malik et al. (1990) described a case with significant decrease in staining of myelin as compared with age-matched, normal control brains. Before the introduction of CT/MRI, other authors also found demyelination of the brain in congenital muscular dystrophy (Fowler and Manson, 1973). However, the lack of progression of white matter hypodensities on successive CT/MRI scans (Egger et al., 1983; Streib and Lucking, 1989; Cook et al., 1992; our study), the absence of progressive neurological signs and symptoms, and the normal MBP findings in CSF in our patients, are all arguments in favour of a non-progressive cerebral white matter disorder. However, long-term follow-up studies and neuropathological studies will be necessary to have more insight in this topic.

The histological findings in the muscle biopsy specimens from CMD patients with (sub)normal intelligence and low density areas in the white matter reported in the literature and the findings in our series (Table 3) did not differ essentially from those described in "pure" CMD, F-CMD and MEB-D (Leyten et al., 1993). In their series, Topaloğlu et al. (1994) found necrosis in 4 patients with white matter hypodensity and not in "pure" CMD. In our series, only fat cell infiltration was found to be increased with increasing age in "pure" CMD (Leyten et al., 1993). Therefore, muscle biopsies cannot discriminate between the subtypes of CMD.

In conclusion, the data published in the literature (Table 3) and our findings support the hypothesis that the "pure" form of CMD with (sub)normal mental development can be subdivided into two subtypes, i.e. those with and those without white matter hypodensities. However, considerable overlap is evident in clinical and pathological presentations. The subtype with white matter hypodensities tend to be clinically more severe (Topaloğlu et al., 1994) and a mild form of epilepsy can be part of the clinical picture. The features are not sufficient to consider this subtype of CMD as a separate entity between the "pure" form of CMD and the Fukuyama type of CMD (Dubowitz, 1994).

Further research should aim to perform more MRI in a prospective longitudinal way, at different ages and on larger groups of children with CMD, and should include neuropathological examination of the hypodense white matter areas. Molecular genetic studies ("genetic mapping") should help to clarify whether the possible etiologic heterogeneity in the three subtypes of CMD is caused by allelic mutations of the same gene or by mutations in different genes. The responsible gene for the Fukuyama type of CMD has been localized at chromosome 9q31-33 (Toda et al., 1993) and we are probably now reaching the stage with CMD having clearly defined basic syndromes.

References

Banker, B.Q. (1986) Congenital muscular dystrophy. In: A.G. Engel and B.Q. Banker (Eds.), Myology, vol. 2, McGraw-Hill, New York, pp. 1367-1382.

Bernier, J.-P., M.H. Brooke, T.P. Naidich and J.E. Carroll (1979) Myoencephalopathy: cerebral hypomyelination revealed by CT scan of the head in a muscle disease. Trans. Am. Neurol. Assoc., 104: 244-246.

Castro-Gago, M. and J. Peña-Guitián (1988) Congenital muscular dystrophy of a non-Fukuyama type with characteristic CT images (letter). Brain Dev., 10: 60.

Cook, J.D., G.G. Gascon, A. Haider, R. Coates, B. Stigsby, P.T. Ozand and M. Banna (1992) Congenital muscular dystrophy with abnormal radiographic myelin pattern. J. Child Neurol., 7(Suppl): 51-63.

Dobyns, W.B., J.B. Kirkpatrick, H.M. Hittner, R.M. Roberts and F.L. Kretzer (1985) Syndromes with lissencephaly. II. Walker-Warburg and cerebro-oculo-muscular syndromes and a new syndrome with type II lissencephaly. Am. J. Med. Genet., 22: 157-195.

Donner, M., J. Rapola and H. Somer (1975) Congenital muscular dystrophy: a clinico-pathological and follow-up study of 15 patients. Neuropädiatrie, 6: 239-258.

Dubowitz, V. (1994) Workshop report: 22nd ENMC sponsored workshop on congenital muscular dystrophy held in Baarn, The Netherlands, 14-16 May 1993. Neuromusc. Disord., 4: 75-81.

Echenne, B., M. Arthuis, C. Billard, J. Campos-Castello, Y. Castel, O. Dulac, D. Fontan, A. Gauthier, S. Kulalowski, G. De Meuron, J.R. Moore, M. Nieto-Barrera, M. Pages, D. Parain, L. Pavone and G. Ponsot (1986) Congenital muscular dystrophy and the cerebral CT scan anomalies. J. Neurol. Sci., 75: 7-22.

Egger, J., B.E. Kendall, M. Erdohazi, B.D. Lake, J. Wilson and E.M. Brett (1983) Involvement of the central nervous system in congenital muscular dystrophy. Dev. Med. Child Neurol., 25: 32-42.

Federico, A., M.T. Dotti, A. Malandrini, G.C. Guazzi, G. Hayek, H. Simonati, N. Rizzuto and P. Toti (1988) Cerebro-ocular dysplasia and muscular dystrophy: report of two cases. Neuropediatrics, 19: 109-112.

Fowler, M. and J.T. Manson (1973) Congenital muscular dystrophy with malformations of the central nervous system. In: B.A. Kakulas (Ed.), Clinical Studies in Myology, ICS 295, Excerpta Medica, Amsterdam, pp. 192-197.

Fukuyama, Y., M. Kawazura and H. Haruna (1960) A peculiar form of congenital progressive muscular dystrophy: report of fifteen cases. Pediatr. Univ. Tokyo, 4: 5-8.

Fukuyama, Y., M. Osawa and H. Suzuki (1981) Congenital progressive muscular dystrophy of the Fukuyama type: clinical, genetic and pathological considerations. Brain Dev., 3: 1-29.

Gubbay, S.S., J.N. Walton and G.W. Pearce (1966) Clinical and pathological study of a case of congenital muscular dystrophy. J. Neurol. Neurosurg. Psychiat., 29: 500-508.

Heggie, P., H.E. Grossniklaus, U. Roessmann, S.M. Chou and R.P. Cruse (1987) Cerebro-ocular dysplasia-muscular dystrophy syndrome: report of two cases. Arch. Ophthalmol., 105: 520-524.

Kaciński, M., B. Bańdo, S. Kroczka and M.M. Malinowska-Matuszewska (1992) A case of congenital muscular dystrophy with changes in the white matter of the brain. Neurol. Neurochir. Pol., 26: 383-387 (in Polish).

Lazaro, R.P., G.M. Fenichel and A.W. Kilroy (1979) Congenital muscular dystrophy: case reports and reappraisal. Muscle Nerve, 2: 349-355.

Leyten, Q.H., F.J.M. Gabreëls, W.O. Renier, H.J. ter Laak, R.C.A. Sengers and R.A. Mullaart (1989) Congenital muscular dystrophy. J. Pediatr., 115: 214-221.

Leyten, Q.H., F.J.M. Gabreëls and W.O. Renier (1990) Classification of congenital muscular dystrophy (letter in reply to H. Topaloğlu, Y. Renda, K. Yalaz, K. Gücüyener, M. Çağlar, S. Göğüs, and G. Kale). J. Pediatr., 117: 166-167.

Leyten, Q.H., K. Renkawek, W.O. Renier, F.J.M. Gabreëls, C.M. Mooy, H.J. ter Laak and R.A. Mullaart (1991) Neuropathological findings in muscle-eye-brain disease (MEB-D): neuropathological delineation of MEB-D from congenital muscular dystrophy of the Fukuyama type. Acta Neuropathol. (Berl.), 83: 55-60.

Leyten, Q.H., F.J.M. Gabreëls, W.O. Renier, K. Renkawek, H.J. ter Laak and R.A. Mullaart (1992) Congenital muscular dystrophy with eye and brain malformations in six Dutch patients. Neuropediatrics, 23: 316-320.

Leyten, Q.H., H.J. ter Laak, F.J.M. Gabreëls, W.O. Renier, K. Renkawek and R.C.A. Sengers (1993) Congenital muscular dystrophy: a study on the variability of morphological changes and dystrophin distribution in muscle biopsies. Acta Neuropathol. (Berl.), 86: 386-392.

Malik, S., R.P. Cruse, S.M. Chou and T. Chafel (1990) Non-Fukuyama congenital muscular dystrophy with central nervous system involvement: clinical, radiographic and pathological correlates (abstract). Ann. Neurol., 28: 430.

Martinelli, P., A.S. Gabellini, G. Ciucci, E. Govoni, S. Vitali and M.R. Gulli (1987) Congenital muscular dystrophy with central nervous system involvement: case report. Eur. Neurol., 26: 17-22.

McKusick, V.A. (1988) Mendelian Inheritance in Man, 8th edn., Johns Hopkins University Press, Baltimore/London, entry 23667.

McMenamin, J.B., L.E. Becker and E.G. Murphy (1982) Congenital muscular dystrophy: a clinicopathologic report of 24 cases. J. Pediatr., 100: 692-697.

Nogen, A.G. (1980) Congenital muscle disease and abnormal findings on computerized tomography. Dev. Med. Child Neurol., 22: 658-663.

Pihko, H., T. Louhimo, L. Valanne and M. Donner (1992) CNS in congenital muscular dystrophy without mental retardation. Neuropediatrics, 23: 116-122.

Raitta, C., M. Lamminen, P. Santavuori and J. Leisti (1978) Ophthalmological findings in a new syndrome with muscle, eye and brain involvement. Acta Ophthalmol. (Copenh.), 56:465-472.

Santavuori, P., H. Somer, K. Sainio, J. Rapola, S. Kruus, T. Nikitin, L. Ketonen and J. Leisti (1989) Muscle-eye-brain disease (MEB). Brain Dev., 11: 147-153.

Serratrice, G., D. Cros, J.-F. Pellissier, J.-L. Gastaut and J. Pouget (1980) Dystrophie musculaire congénitale. Rev. Neurol. (Paris), 136: 445-472.

Streib, E.W. and C.H. Lucking (1989) Congenital muscular dystrophy with leukoencephalopathy. Eur. Neurol., 29: 211-215.

Tanaka, J., T. Mimaki, S. Okada and H. Fujimura (1990) Changes in cerebral white matter in a case of congenital muscular dystrophy (non-Fukuyama type). Neuropediatrics, 21: 183-186.

Toda, T., M. Segawa, Y. Nomura, I. Nonaka, K. Masuda, T. Ishihara, M. Suzuki, I. Tomita, Y. Origuchi, K. Ohno, N. Misugi, Y. Sasaki, K. Takada, M. Kawai, K. Otani, T. Murakami, K. Saito, Y. Fukuyama, T. Shimizu, I. Kanazawa and Y. Nakamura (1993) Localization of a gene for Fukuyama type congenital muscular dystrophy to chromosome 9q31-33. Nature Genet., 5: 283-286.

Topaloğlu, H., G. Kale, D. Yalnizoğlu, A.H. Taşdemir, A. Karaduman, M. Topçu and E. Kotiloğlu (1994) Analysis of "pure" congenital muscular dystrophies in thirty-eight cases. How different is the classical type 1 from the occidental type cerebromuscular dystrophy? Neuropediatrics, 25: 94-100.

Towfighi, J., J.W. Sassani, K. Suzuki and R.L. Ladda (1984) Cerebro-ocular dysplasia-muscular dystrophy (COD-MD) syndrome. Acta Neuropathol. (Berl.), 65: 110-123.

Vassella, F., M. Mumenthaler and E. Rossi (1970) Congenital muscular dystrophy. In: J.N. Walton, N. Canal and G. Scarlato (Eds.), Muscle Disease, Proc. Int. Cong, Milan, May 19-21, 1969, ICS 199, Excerpta Medica, Amsterdam, pp. 620-623.

Yoshioka, M., S. Kuroki and H. Mizue (1987) Congenital muscular dystrophy of non-Fukuyama type with characteristic CT images. Brain Dev., 9: 316-318.

Congenital muscular dystrophy with eye and brain malformations in six Dutch patients

Q.H. Leyten, F.J.M. Gabreëls, W.O. Renier,
K. Renkawek, H.J. ter Laak, R.A. Mullaart

Neuropediatrics 1992;23:316-320

Abstract

From four Dutch families six patients, who have congenital muscular dystrophy, involvement of the central nervous system and of the eyes, or the so-called "muscle, eye and brain disease" (MEB-D), are reported. Two patients are still alive, in four autopsy could be performed. The clinical and morphological data of our patients are compared to those described in recent literature. The progression of the disease was rapid in five of our six patients. Our study supports the idea that within the MEB-D syndrome there are at least two different types of clinical expression, one with a rapid progression as described by *Dobyns* et al (1989) and one with a slower progression as described in most patients of *Santavuori* et al (1989). The study also confirms the autosomal recessive mode of inheritance of MEB-D.

The term "muscle, eye and brain disease" (MEB-D) was first introduced by *Santavuori* et al (22) and *Raitta* et al (21), representing a rare autosomal recessive disorder characterized by muscular dystrophy, visual failure, mental retardation, macrocephaly and often severe hydrocephalus

After the discovery of muscle involvement in the *Walker-Warburg* syndrome, previously known to consist of hydrocephalus, severe mental retardation and ocular malformation (15, 26), most authors actually agree that both conditions form one and the same entity (9, 10, 17, 18, 20-25) A consensus of this opinion was recently accepted at the VII International Congress on Neuromuscular Disease, Munich, FRG, 16-22 September 1990

Dobyns et al (9) have proposed four diagnostic criteria for "muscle, eye and brain disease" (1) congenital muscular dystrophy, (2) retinal malformation, (3) cerebral malformation, type II lissencephaly or variants, and (4) cerebellar malformation Hydrocephalus and anterior chamber malformation of the eye are two other major abnormalities, which are not constant but very frequent

In this paper we describe six Dutch patients with MEB-D Four patients have yet been mentioned in a previous survey of congenital muscular dystrophy (CMD) (17) Two new cases are added The clinical expression, follow-up and morphological data of these six patients are compared with recent descriptions of MEB-D in the literature (1-9, 12-16, 23, 26-30)

Clinical features (Table 1)

Two children were born by breech delivery Mild postnatal asphyxia occurred in three cases All had hypotonia and generalized weakness from birth In most cases, proximal muscles were more affected than distal ones and, in general, the arms were more impaired than the legs Contractures were present at birth in two cases All patients had severe involvement of the central nervous system (CNS) and the eyes, and appeared profoundly retarded One patient had left-sided hemiconvulsions, which were treated successfully with anticonvulsive therapy Three patients had microcephaly and the other three had macrocephaly In three patients, an occipital encephalocele was present

Ophthalmological examination revealed microphthalmia and blepharophimosis of the right eye (Fig 1) and clouding of the cornea in Cases 1 and 2, who were sisters, of whom one also had choreoid atrophy and retinal detachment of the left eye and of whom the other had fetal anterior chamber angle of the left eye Case 3 had an antimongoloid position of the eyes with cataract and iris atrophy, and the cornea of the right eye had an opaque area in the central part Ocular

Table 1. Diagnostic criteria, signs and symptoms, laboratory, radiological and neurophysiological characteristics of six Dutch cases with "muscle, eye and brain disease" compared to the data of the cases of *Dobyns* (9) and of *Santavuori* (23)

	Our cases (n = 6)	*Dobyns*' cases (n = 21)	*Santavuori*'s cases (n = 19)
Clinical expression			
Perinatal asphyxia	3/6	often	0/19
Congenital hypotonia	6/6	21/21	10/19 (19/19)[a]
Early pyramidal signs	4/6	–	>5 yr
Early joint contractures	2/6	7/20	0/19
Age at death	2d - 5yr (4 cases)	mean 2 mo (19 cases)	6-16 yr (4 cases)
Age of survivors	4 yr, 11 yr	5-10% >5 yr	(2.5)-46 yr
Muscle			
Increased CK activity	6/6	8/8	0/19 (19/19)[b]
Myogenic pattern of EMG	6/6	2/2	14/14[c]
Dystrophic muscular biopsy	5/5	14/14	16/16[d]
Normal distribution of dystrophin	2/2	–	–

OFC = occipital frontal circumference
[a] initially 10/19, after 6 months 19/19
[b] initially normal, later increased in 19/19
[c] initially normal, after 1-2 years myopathic in 14/14
[d] initially normal, after 6 months dystrophic in 16/16

Table 1. (continued)

	Our cases (n = 6)	*Dobyns'* cases (n = 21)	*Santavuori's* cases (n = 19)
Eye			
Ophthalmological disorder	6/6	21/21	19/19
Brain			
CNS involvement	6/6	21/21	19/19
– mental retardation			
– mild	–	–	2/19
– severe	6/6	21/21	17/19
– OFC			
– congenital macrocephaly	3/6	11/19	–
– congenital microcephaly	3/6	3/19	–
– encephalocele	3/6	5/21	0/19
– hydrocephalus	5/6	20/21	11/19
– hypomyelination	1/6	11/21	7/21
– cerebellar malformation	4/6	20/20	slight atrophy in 11/19

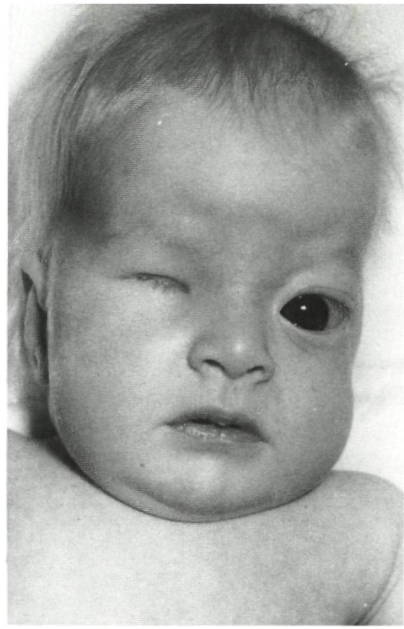

Fig. 1. Case 1. Microphthalmia and blepharophimosis of the right eye.

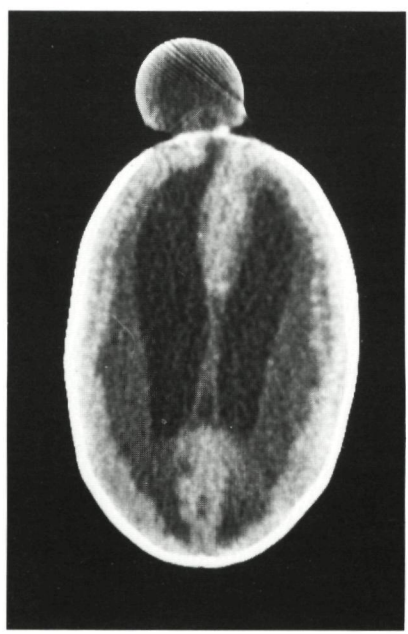

Fig. 2. Case 4. Enlarged lateral ventricles and occipital encephalocele.

involvement in Case 4 revealed fetal anterior chamber angle in the left eye, cataract in the right eye, oblique implantation of the papil with excavation as seen in glaucoma. Case 5 had pendicular horizontally nystagmus and uncontrolled eye movements of central origin. The fundus showed a white coloured retina and a white coloured atrophic macula with pigmentation. In Case 6 a subcapsular and polaris posterior cataract in both eyes and strabismus convergens appeared to be present.

No patient did reach any motor milestones. Four patients died at the ages of 2 days, 2 months, 3 months and 5 years, respectively.

Genetical aspects

In our series there are four girls and two boys. There are two sets of familial cases: two sisters (Cases 1 and 2), and a brother and sister (Cases 3 and 4). In only one case (Case 5) consanguinity was proved. In all cases chromosomal examination revealed a normal pattern.

Laboratory investigations

Appropriate studies ruled out inborn errors of metabolism, endocrinological, immunological and chronic infectious diseases, vitamin deficiencies, and disorders caused by toxic agents. All six patients had increased CK activity (between 990 U/l and 1540 U/l, normal < 90 U/l).

Neurophysiological investigations

Electroencephalography in the examined cases showed diffuse slowing and paroxysmal discharges. Visual evoked potentials and brain stem auditory evoked potentials were examined in two cases and appeared severely disturbed. Electromyographic studies indicated a severe myopathic trace in all patients, while motor nerve conduction velocities were normal.

Radiological investigations

Computerized tomography of the brain could be performed in five of the six children and showed lissencephaly and enlarged third and lateral ventricles (hydrocephaly) in all five. In Case 6 irregular porencephaly was present in the left frontal lobe. Three had an occipital encephalocele (Fig. 2).

Muscle biopsies

Light microscopical examination of muscle biopsy or autopsy samples from five out of six patients was performed and revealed dystrophic changes ranging from

slight to very severe. The least affected biopsy showed a slight increase of fat cells and connective tissue, and a slightly increased variability of muscle fibre size. In the most affected specimen, almost all muscle fibres were replaced by fat cells. In all specimens, rounded fibres were present and in all cases there was an increased variation in fibre size diameter caused by the presence of atrophic and hypertrophic fibres (Fig. 3). Fibres with internal nuclei were observed in one case, basophilic fibres in two cases, some hyaline fibres in three cases and sporadic necrotic fibres in one case. In two cases, immunohistochemical investigations for the presence of dystrophin could be performed; normal distribution patterns were found.

Biochemical studies

Pyruvate oxidation and fatty acid oxidation were in the normal range. Carnitine content was examined in three cases and revealed no abnormalities.

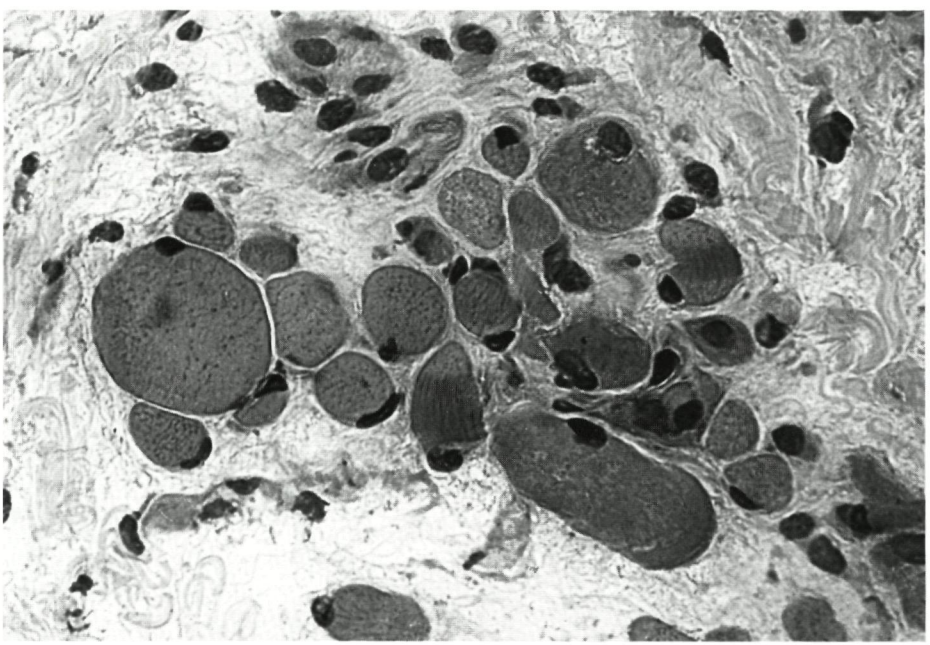

Fig. 3. Dystrophic picture of the soleus muscle with rounded muscle fibres of varying diameter and clear increase of connective tissue, Haematoxylin and Eosin, x 400.

Neuropathological examination

In four cases autopsy has been performed immediately after death. It concerns Case 1 and her sister (Case 2), Case 3 and her brother (Case 4). Two of our patients are still alive. Macroscopical and microscopical examination showed similar picture of brain abnormalities in all cases examined. There was lissencephaly, hydrocephalus, agenesis of the olfactory bulbs, optic chiasma and septum pellucidum, cerebellar hypoplasia, agenesis of vermis and cystic malformation. Light microscopical examination revealed a type II lissencephaly, hypomyelination of cerebral white matter, diffuse leptomeningeal and glial proliferation, disorganization of the cerebral and cerebellar lamination and heterotopia of *Purkinje* cells (Figs. 4 and 5).

Examination of the eyes showed malformations consisting of diffuse disruption of laminar disorder and apparently dysplastic changes due to defective fetal development. Detailed examination of neuropathological and ophthalmological abnormalities of Cases 1 and 2 has been described in our previous publication (18).

Pathological examination

General autopsy of Case 1 revealed several small follicle cysts in the right ovarium. In the adrenals some nodular hyperplasia occurred. Histological examination of the skin excluded ectodermal dysplasia. In Case 2, autopsy of the body revealed slight splenomegaly of the weight (250 g, normal 160 g) and infectious changes in the spleen. In Cases 3 and 4, the lungs and heart were of normal weight and of normal appearance. Cardiomyopathy was not found. Especially renal dysplasia and imperforate anus did not occur.

Discussion

The six Dutch patients manifest the association of congenital cerebro-ocular malformations and dystrophic changes of the muscles, the so-called "muscle, eye and brain disease" (MEB-D) (9, 17, 18, 20-23, 25).

In the literature, two large series on MEB-D have recently been published, one by *Dobyns* et al (9), the other by *Santavuori* et al (23). The former series contains a large survey of the literature (1-8, 12-16, 26-30).

Considering the clinical data in our study, the high rate of respiratory difficulties in half of our cases is remarkable and is also found in *Dobyns'* study (9). In general, the clinical expression of the disease at birth (as perinatal asphyxia,

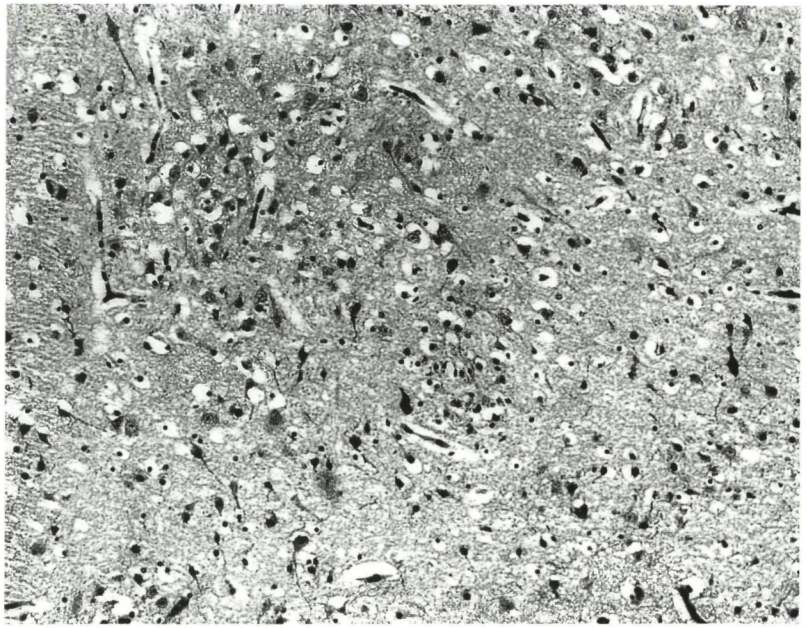

Fig. 4. Disorder of cortical lamination and neural orientation, nest of cells divided by acellular tissue. *Klüver-Barrera*, x 200.

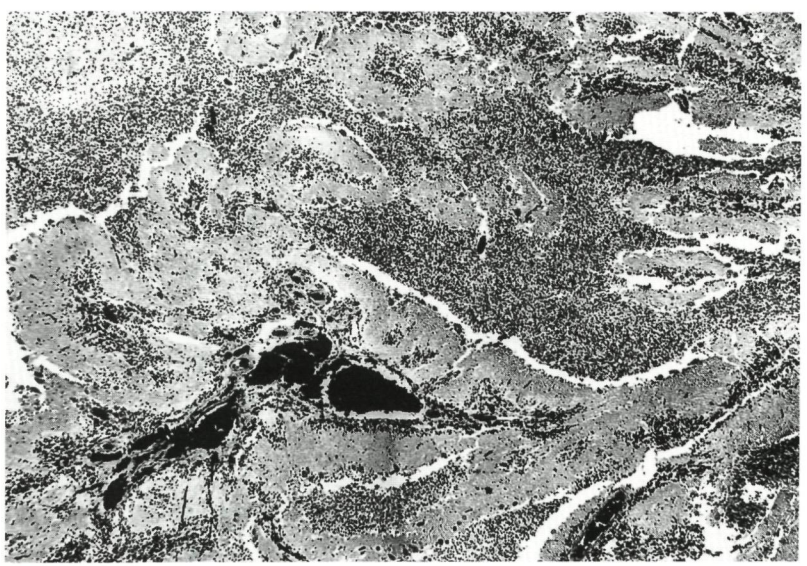

Fig. 5. Disarrangement of cerebellar architecture, proliferation of mesenchymal tissue. Haematoxylin and Eosin, x 120.

congenital hypotonia, early pyramidal signs and early joint contractures) is more severe in our group and in most patients of *Dobyns* (9) compared to those of *Santavuori* (23). Progression of the disease was rapid in five of our six patients, in all patients described by *Dobyns* et al (9) and in most of the patients described in the literature (1-8, 12-16, 26-30). In contrast, patients in *Santavuori*'s study show a slow progression (23). In our study only one patient has a mild course. From the literature study one can expect that 5-10% of the MEB-D patients will have a slow progression (1-9, 12-16, 26-30). Our patients are profoundly retarded, as most in the literature (9). However, mental retardation is mild in two patients of *Santavuori* (23). The initially normal and later increasing CK values in *Santavuori*'s patients (23) contrast with the initially increased CK values in our study and in *Dobyns*' report (9).

Dystrophic characteristics of the muscle generally depend on the stage of the disorder and are usually progressive (11, 17). The dystrophic changes of muscle biopsy in the cases of *Santavuori*'s study (23) seem less severe than in the cases of *Dobyns* (9) and in ours. The histological findings described in *Santavuori*'s study (23) were very variable, ranging from nearly normal to severely dystrophic and the histological picture of a burned out myopathy was not seen even in the oldest patients.

The ophthalmological signs in MEB-D include both functional disturbances, such as nystagmus and uncontrolled eye movements of central origin, and structural eye changes, such as microphthalmia, cleavage defects of the anterior chamber resulting in congenital glaucoma, optic cup deformation resulting in severe myopia, cataract, and retinal changes, such as retinal dysplasia and detachment (9, 17, 20-23, 25). Comparing the ophthalmological characteristics between the three studies (ours, 9, 23) and the other reports from the literature (1-8, 12-16, 26-30) no essential differences have been noticed.

Regarding the neuropathological findings, no essential pathological differences can be found between the patients of *Dobyns*' report (9) and our patients. Encephalocele, cerebellar malformation and hydrocephalus are observed more often in our and in *Dobyns*' study (9), and not or less frequently in the study of *Santavuori* (23). Microscopical data have scarcely been described, but the cerebral gyral anomalies are more widespread in *Dobyns*' group (10).

Considering the clinical expression and progression, and the laboratory, radiological and morphological characteristics in the different studies, we conclude that within the spectrum of "muscle, eye and brain disease" different severities of the disease exist, ranging from a more milder type in the Finnish group (23) to a more severe type in *Dobyns*' group (9) and in most of our patients. Besides

the clinical spectrum of MEB-D our study also confirms the autosomal recessive inheritance of MEB-D (19).

Regarding genetic aspects of MEB-D in our cases and in literature (1-8, 12-16, 26-30), interfamilial differences are more pronounced than intrafamilial, which is compatible with genetic heterogeneity and the possible existence of allelic mutations. Whether this possible etiologic heterogeneity is caused by allelic mutations or by mutations in different genes remains to be elucidated (10, 17, 18).

References

[1] *Attia, M.F.D., J. Burn, J.H. McCarthy, D.P. Purohit, D.W.A. Milligan:* Warburg (HARD +/- E) syndrome without retinal dysplasia: Case report and review. Br. J. Ophthalmol. 70 (1986) 742-747

[2] *Aymé, S., J.F. Mattei:* HARD (+/- E) syndrome: Report of a sixth family with support for autosomal recessive inheritance. Am. J. Med. Genet. 14 (1983) 759-766

[3] *Bordarier, C., J. Aicardi, F. Goutières:* Congenital hydrocephalus and eye abnormalities with severe developmental brain defects: Warburg's syndrome. Ann. Neurol. 16 (1984) 60-65

[4] *Burton, B.K., R.G. Dillard, R.G. Weaver:* Brief clinical report: Walker-Warburg syndrome with cleft lip and cleft palate in two sibs. Am. J. Med. Genet. 27 (1987) 537-541

[5] *Chan, C.C., P.R. Egbert, M.K. Herrick, H. Urich:* Oculocerebral malformations: A reappraisal of Walker's lissencephaly. Arch. Neurol. 37 (1980) 104-108

[6] *Chemke, J., B. Czernobilsky, G. Mundel, Y.R. Barishak:* A familial syndrome of central nervous system and ocular malformations. Clin. Genet. 7 (1975) 1-7

[7] *Crowe, C., M. Jassam, L. Dickerman:* The prenatal diagnosis of the Walker-Warburg syndrome. Prenat. Diagn. 6 (1986) 177-185

[8] *Dambska, M., K. Wisniewski, J. Sher, G. Solish:* Cerebro-oculo-muscular syndrome: A variant of Fukuyama congenital cerebro-muscular dystrophy. Clin. Neuropathol. 1 (1982) 93-98

[9] *Dobyns, W.B., R.A. Pagon, D. Armstrong, C.J.R. Curry, F. Greenberg, A. Grix, L.B. Holmes, R. Laxova, V.V. Michels, M. Robinow, R.L. Zimmerman:* Diagnostic criteria for Walker-Warburg syndrome. Am. J. Med. Genet. 32 (1989) 195-210

[10] *Dobyns, W.B., R.A. Pagon, C.J.R. Curry, F. Greenberg:* Regarding Walker-Warburg syndrome and muscle-eye-brain disease (letter to the editor: response to Santavuori et al). Am. J. Med. Genet. 36 (1990) 373-374

[11] *Donner, M., J. Rapola, H. Somer:* Congenital muscular dystrophy: A clinicopathological and follow-up study of 15 patients. Neuropädiatrie 6 (1975) 239-258

[12] *Halsey, J.H., N. Allen, H.R. Chamberlin:* The morphogenesis of hydranencephaly. J. Neurol. Sci. 12 (1971) 187-217

[13] *Heggie, P., H.E. Grossniklaus, U. Roessmann, S.M. Chou, R.P. Cruse:* Cerebro-ocular dysplasia-muscular dystrophy syndrome: Report of two cases. Arch. Ophthalmol. 105 (1987) 520-524

[14] *Korinthenberg, R., D. Palm, W. Schlake, J. Klein:* Congenital muscular dystrophy, brain malformation and ocular problems (muscle, eye and brain disease) in two German families. Eur. J. Pediatr. 142 (1984) 62-68

[15] *Krause, A.C.:* Congenital encephalo-ophthalmic dysplasia. Arch. Ophthalmol. 36 (1946) 387-414

[16] *Krijgsman, J.B., P.G. Barth, F.C. Stam, J.L. Slooff, H.H.J. Jaspar:* Congenital muscular dystrophy and cerebral dysgenesis in a Dutch family. Neuropädiatrie 11 (1980) 108-120

[17] *Leyten, Q.H., F.J.M. Gabreëls, W.O. Renier, H.J. ter Laak, R.C.A. Sengers, R.A. Mullaart:* Congenital muscular dystrophy. J. Pediatr. 115 (1989) 214-221

[18] *Leyten, Q.H., K. Renkawek, W.O. Renier, F.J.M. Gabreëls, C.M. Mooy, H.J. ter Laak, R.A. Mullaart:* Neuropathological findings in muscle-eye-brain disease (MEB-D): Neuropathological delineation of MEB-D from congenital muscular dystrophy of the Fukuyama type. Acta Neuropathol. 83 (1991) 55-60

[19] *McKusick, V.A.:* Mendelian inheritance in Man. Baltimore, Johns Hopkins University Press (1986) no. 25328

[20] *Pavone, L., F. Gullotta, S. Grasso, C. Vannucchi:* Hydrocephalus, lissencephaly, ocular abnormalities and congenital muscular dystrophy: A Warburg syndrome variant? Neuropediatrics 17 (1986) 206-211

[21] *Raitta, C., M. Lamminen, P. Santavuori, J. Leisti:* Ophthalmological findings in a new syndrome with muscle, eye and brain involvement. Acta Ophthalmol. (Copenh) 56 (1978) 465-472

[22] *Santavuori, P., J. Leisti, S. Kruus:* Muscle, eye and brain disease: A new syndrome (abstract). Neuropädiatrie 8 (Suppl) (1977) 553

[23] *Santavuori, P., H. Somer, K. Sainio, J. Rapola, S. Kruus, T. Nikitin, L. Ketonen, J. Leisti:* Muscle-eye-brain disease (MEB). Brain Dev. 11 (1989) 147-153

[24] *Santavuori, P., H. Pihko, K. Sainio, M. Lappi, H. Somer, M. Haltia, C. Raitta, L. Ketonen, J. Leisti:* Muscle-eye-brain disease and Walker-Warburg syndrome (letter to the editor). Am. J. Med. Genet. 36 (1990) 371-372

[25] *Towfighi, J., J.W. Sassani, K. Suzuki, R.L. Ladda:* Cerebro-ocular dysplasia-muscular dystrophy (COD-MD) syndrome. Acta Neuropathol. (Berl) 65 (1984) 110-123

[26] *Walker, A.E.:* Lissencephaly. Arch. Neurol. Psychiatr. 48 (1942) 13-29

[27] *Whitley, C.B., T.R. Thompson, A.R. Mastri, R.J. Gorlin:* Warburg syndrome: Lethal neurodysplasia with autosomal recessive inheritance. J. Pediatr. 102 (1983) 547-551

[28] *Williams, R.S., C.N. Swisher, M. Jennings, M. Ambler, V.S. Caviness Jr.:* Cerebro-ocular dysgenesis (Walker-Warburg syndrome): Neuropathologic and etiologic analysis. Neurology 34 (1984) 1531-1541

[29] *Winter, R.M., A. Gardner:* Hydrocephalus, agyria, pseudoencephalocele, retinal dysplasia, and anterior chamber anomalies. J. Med. Genet. 18 (1981) 314-317

[30] *Yanoff, M., L.B. Rorke, M.I. Allman:* Bilateral optic system aplasia with relatively normal eyes. Arch. Ophthalmol. 96 (1978) 97-101

MORPHOLOGICAL STUDIES

Congenital muscular dystrophy

A study on the variability of morphological changes and dystrophin distribution in muscle biopsies

Q.H. Leyten, H.J. ter Laak, F.J.M. Gabreëls,
W.O. Renier, K. Renkawek, R.C.A. Sengers

Acta Neuropathologica 1993;86:386-392

Abstract

Histomorphological and histochemical variability was studied in muscle specimens from 30 patients with congenital muscular dystrophy (CMD). We found involvement of the central nervous system in 8 patients (Fukuyama CMD, F-CMD), involvement of the brain and the eyes in 5 patients (muscle, eye and brain disease, MEB-D) and hypodense white matter on the CT scans of 2 patients with (sub)normal intelligence (occidental-type cerebromuscular dystrophy, O-CMD). No morphological hallmarks were found to differentiate these subgroups. Only fat cell infiltration was found to be increased with increasing age in 'pure' CMD (pure-CMD). The morphological data did not appear to be correlated with the clinical severity or type of dystrophy (pure-CMD, F-CMD, MEB-D and O-CMD). Immunohistochemistry with dystrophin, vimentin and desmin antibodies in 14 patients (6 pure-CMD, 5 F-CMD, 2 MEB-D and 1 O-CMD) showed a normal expression pattern.

Congenital muscular dystrophy (CMD) encompasses several groups of congenital degenerative muscle diseases with great variation in clinical appearance [2, 4, 7-14, 17-20, 22, 28, 30-33, 35, 38]. We can distinguish 'pure' CMD (pure-CMD), CMD with normal (or subnormal) intelligence and hypodense white matter on CT scans, also called 'Occidental-type CMD' (O-CMD) [34], CMD with malformation of the central nervous system or 'Fukuyama-CMD' (F-CMD) and CMD with involvement of the central nervous system and the eyes, so-called 'muscle, eye and brain disease' (MEB-D) [9, 30-33, 35].

Dystrophin distribution has been described in a few cases of pure-CMD [1, 24, 29, 36, 37] and F-CMD [1, 27]. As the distribution was found to be disturbed in some of the studies on F-CMD [1, 27], we compared our results to those studies.

The aim of this study was (i) to investigate whether there are any qualitative and/or quantitative morphological differences between the muscle biopsies of patients suffering from pure-CMD, O-CMD, F-CMD and MEB-D, (ii) to present and discuss the results of dystrophin, desmin, and vimentin distribution in these patients, and (iii) to investigate whether there is a possible relation between muscle pathology and the clinical severity of the disease.

Material and Methods

From the 2010 muscle biopsies (performed and stained according to standard procedures) examined at the neuromorphological department over the past 18 years, 30 were diagnosed as having CMD. The final clinical diagnosis of pure-CMD, O-CMD, F-CMD or MEB-D was based on clinical data and CT/magnetic resonance imaging. The clinical severity of CMD was expressed by the individual motor development of the patients (Fig. 1).

Clinical data (Table 1, Fig. 1)

Some patients showed mild postnatal asphyxia. Generalized hypotonia and weakness from birth with significantly delayed motor development (Fig. 1) was noted in all patients. In most patients, proximal muscles were more affected than distal ones and generally the arms were more impaired than the legs. Contractures were often observed (at birth or later). CNS involvement included mild or severe mental retardation, epilepsy, microcephaly, macrocephaly, encephalocele, meningocele, and hydrocephalus. Ophthalmological involvement included microphthalmus, corneal clouding, fetal anterior chamber angle,

Table 1. Diagnostic criteria, signs, symptoms, laboratory, radiological, and neurophysiological characteristics of 30 patients with congenital muscular dystrophy (CMD)

	Pure-CMD $n = 15$	Occidental-type CMD $n = 2$	Fukuyama-CMD $n = 8$	Muscle-eye-brain disease $n = 5$
Clinical expression				
Perinatal ashyxia	3/15	0/2	2/8	2/5
Congenital hypotonia	15/15	2/2	8/8	5/5
Joint contractures	10/15	1/2	4/8	2/5
Rapidly progressive course	4/15	1/2	4/8	4/5
Muscle				
Increased CK level	6/15	1/2	4/8	5/5
Myogenic pattern of EMG	15/15	2/2	7/7	5/5
Dystrophic muscular biopsy	15/15	2/2	8/8	5/5
Normal distribution of dystrophin	6/6	1/1	5/5	2/2
Eye				
Ophthalmological disorders	0/15	0/2	0/8	5/5
Brain				
CNS involvement	0/15	2/2	8/8	5/5
Mental retardation	0/15	0/2	8/8	5/5
Congenital macrocephaly	0/15	0/2	0/8	2/5
Congenital microcephaly	0/15	0/2	3/8	2/5
Encephalocele	0/15	0/2	0/8	3/5
Hydrocephalus	0/15	0/2	4/8	4/5
Hypomyelination	0/15	2/2	2/8	1/5
Cerebellar malformation	0/15	0/2	3/8	3/5

macular atrophy, severe myopia, and uncontrolled eye movements. All our patients had normal or moderately increased CK levels, the highest ones being present in the MEB-D group (see Table 2). For more details we refer to our papers on congenital muscular dystrophy [19] and on neuropathological findings in "muscle-eye-brain disease" [20].

Postmortem examination

Seven patients have died (see Fig. 1). Five brains were available for postmortem examination (two F-CMD and three MEB-D). The data of one F-CMD patient (no. 20) and two MEB-D patients (no. 26 and 27, who were sisters) have already been described, [17] and [20], respectively. Generally, neuropathology was characterized by severe hydrocephalus and defective brain development; low density of the cerebral white matter in CT scan was common.

One F-CMD brain (no. 20) revealed a lissencephalic and partly polymicrogyric neocortex, absent corpus callosum and a bridge of gray matter linking both hemispheres, absence of cerebral cortex lamination with subcortical neuronal heterotopic masses, white matter gliosis, a polymicrogyric cerebellum and a true hydrocephalus presumably due to aqueductal stenosis. The other F-CMD brain (no. 19) was microcephalic and showed severe depletion of neurons, demyelination and neuronal migration defect. Cortical layering was absent, and gray and white matter were poorly delineated. Further, this brain showed a thin corpus callosum, hydrocephalus and diffuse poverty of myelin fibers.

The pathological findings in MEB-D (no. 26, 27 and 30) were lissencephaly, hydrocephalus, absence of cerebral cortex lamination, hypomyelination, heterotopia, Dandy-Walker cysts, and agenesis of olfactory and optic tracts, septum, corpus callosum, and vermis. Examination of the eyes was performed in one patient (no. 26) and revealed changes due to defective eye development of fetal origin. Both eyes were microphthalmic and among others revealed retinal dysplasia, fetal chamber, coloboma, and cataract.

Morphological biopsy parameters

In all of the muscle biopsies (mostly taken from the quadriceps muscle), the following parameters were semiquantitatively examined and expressed in relation to their severity (–, normal or absent; +/–, present; +, increased; ++, marked; and +++, extensive): the presence of fat cell infiltration, endomysial connective tissue, fibers with internal nuclei, basophilic fibers, necrotizing or necrotic fibers, atrophy of type I fibers and type II fibers and hypertrophy of type I and type II fibers; the presence of type I and type IIC fibers was also expressed.

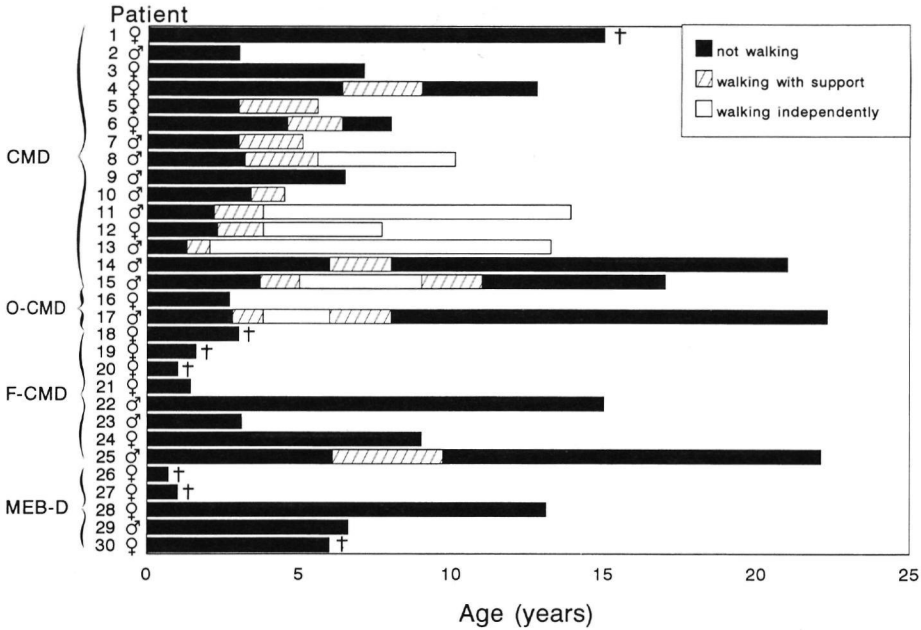

Fig. 1. Motor development of 15 patients with pure congenital muscular dystrophy (CMD), 2 with occidental type (O)-CMD, 8 with Fukuyama (F)-CMD, and 5 with muscle-eye-brain disease (MEB-D). Age of ambulation at the end of follow-up or at death (†).

Immunohistochemical labelling of dystrophin, desmin, and vimentin

Immunohistochemical labelling of dystrophin, vimentin and desmin was carried out on six pure-CMD patients, five F-CMD patients, two MEB-D patients and one O-CMD patient. Unfixed frozen sections, 8 μm thick, were cut using a cryostat microtome. These sections were mounted directly onto microscope coverslips and stored at −30°C until required. Immunolabelling was done by incubating (1 h) sections with primary monoclonal antibody against the C terminus of dystrophin (DYS 2, Novocastra Laboratories) diluted with phosphate-buffered saline (PBS 1:2). After rinsing in PBS (3 x 3 min), peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dakopatts) were added and left for 1 h (dilution 1:10). Immunohistochemical labelling of desmin and vimentin was also performed by incubating sections with diluted (1:40) polyclonal antibodies against desmin and vimentin (both from Euro-diagnostics) for 1 h; for these antibodies peroxidase-conjugated goat anti-rabbit immunoglobulins (Nordic) were used. Sections were washed again in PBS (3 x 3 min) prior to evaluation of the peroxidase label by using a solution with 3-amino-9-ethyl-

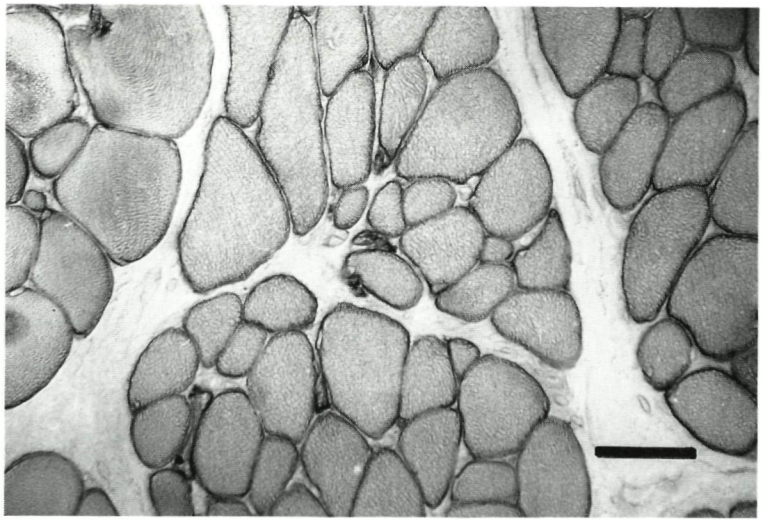

Fig. 2. Dystrophin immunostain of case 9 (pure-CMD). Note the even distribution on both small and hypertrophic muscle fibers. Bar = 50 μm.

carbazole (for dystrophin) or 3,3'-diaminobenzidine tetrahydrochloride (for desmin and vimentin) for 5 min. To avoid misinterpretation, immunolabelling of control tissue sections from other patients - of the same age and stored for the same period - was also performed simultaneously.

Statistical analysis

Wilcoxon's test (or modifications of this test) or exact calculation of the results was performed to determine *P* values (a value smaller than 0.05 or exceeding 0.95 was considered to be significant).

Results

Histometry

Patients were classified according to their final clinical diagnosis and arranged according to their age (Table 2). From this table it can be seen that there was considerable variability in the observed parameters. After statistical testing no significant differences were found between pure-CMD, O-CMD, F-CMD and MEB-D, except for the increase in fat cell infiltration with age in pure-CMD.

Table 2. Morphological parameters in pure-CMD, occidental type CMD, Fukuyama-CMD and muscle-eye-brain disease

	Pure-CMD in patients up to 3 years of age						Pure-CMD in patients of 3 years of age and older									Occidental type CMD	
Patient no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Age of examination (years)	0	0	1	1	1	2	3	3	3	3	4	5	6	7	14	1	6
Sex	F	M	F	F	F	M	M	M	M	M	M	M	M	M	M	F	M
Biopsy location	Q	Q	Q	Q	Q	Q	Sol	Q	RA	Q	Q	Q	Q	Q	Q	Q	Q
CK activity (normal <90 U/l)	288	N	N	N	33	N	18	143	N	N	134	153	392	282	N	117	400
Fat	+	–	–	–	–	–	+++	+++	–	+	++	++	+	++	+++	+	+++
Connective tissue	++	+	+	+	–	++	++	++	++	+	+	+	++	++	++	++	+
Internal nuclei	–	–	–	–	–	++	++	–	–	–	+	–	+	++	++	+	+
Basophilic fibers	+	–	+	+	–	–	++	+	–	+	++	+	–	+	–	–	+
Necrotic fibers	–	–	–	–	–	–	–	–	–	+	+	–	+	+	–	+	+
Hyaline fibers	–	–	+/–	+/–	–	–	+/–	+/–	–	–	+/–	–	–	+/–	–	–	–
Percentage type I fibers	NO	48	34	55	67	99	87	30	70	55	42	82	54	51	50	76	77
Type IIC fibers	NO	–	–	+	–	–	+	–	–	+	+++	++	–	++	–	+	+
Atrophic fibers																	
– type I	++	+++	++	++	++	+	++	+	+	+	+++	+	–	+	–	+	+
– type II		+++	++	++	+	+	+	+	+	+++	+++	+	–	+	+++	–	–
Hypertrophic fibers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
– type I	+	–	–	++	+	++	+	+	+	+++	++	–	++	++	+++	++	+++
– type II		+++	–	++	+	–	++	+	+	+	++	–	++	++	–	++	+++
Dystrophin distribution	NO	N	NO	NO	N	NO	N	NO	N	N	NO	N	NO	NO	NO	N	NO
Desmin/Vimentin	NO	N	NO	NO	N	NO	N	NO	N	N	NO	N	NO	NO	NO	N	NO

Table 2. (continued)

	Fukuyama-CMD										Muscle-eye-brain disease				
Patient no	18A[a]	18B[a]	18C[a]	19	20	21	22	23	24	25	26	27	28	29	30
Age of examination (years)	0	1	1	0	0	1	2	3	4	10	0	0	0	5	5
Sex		F		F	M	F	M	M	F	M	F	F	F	M	F
Biopsy location	Q	Sol	Q	Q	Q	Sol	Q	Q	Q	Q	Q	Q	Sol	Q	Q
CK activity (normal <90 U/l)		590		527	690	37	N	60	192	N	1130	1540	1495	900	990
Fat	–	+++	+++	–	–	+	++	+++	–	++	+	+	+	+	++
Connective tissue	+	++	+	+	++	+	+++	+++	+	+	+	+	++	+	++
Internal nuclei	–	+	+	–	+	–	+	++	–	+	–	–	–	–	+
Basophilic fibers	++	+	+	+	+	–	+	–	–	–	NO	NO	++	+	–
Necrotic fibers	+	–	–	–	+	–	–	–	–	–	–	–	–	+/–	–
Hyaline fibers	+/–	–	–	–	++	–	+/–	–	–	–	+/–	+/–	+/–	–	–
Percentage type I fibers	90	40	77	40	30	97	36	100	53	64	NO	NO	40	59	40
Type IIC fibers	–	+	–	++	+++	–	+++	–	–	–	NO	NO	–	–	NO
Atrophic fibers															
– type I	+	+	+	+	++	+	++	++	–	+	+	+	+	+	+
– type II	+	+	+	+	++	+	++	+++	–	+			+	–	+
Hypertrophic fibers															
– type I	+	–	–	+	++	–	+	NO	+	++	++	++	+	–	–
– type II	+	–	+	–	++	–	+	NO	+	++			+	–	+
Dystrophin distribution	NO	N	N	N	NO	N	NO	N	N	NO	NO	NO	NO	N	N
Desmin/Vimentin	NO	N	N	N	NO	N	NO	N	N	NO	NO	NO	NO	N	NO

F, female; M, male; N, normal; A, abnormal; NO, not observed or not performed, Q, quadriceps muscle; Sol, soleus muscle, RA, rectus abdominis muscle; –, normal or absent; +/–, present; +, increased; ++, marked, +++, extensive

[a] Biopsies from the same patient

Table 3. Studies of dystrophin in patients with pure-CMD, Fukuyama-CMD, occidental type CMD, and muscle-eye-brain disease

CMD type	Pure-CMD		Occidental-type CMD		Fukuyama-CMD		Muscle-eye-brain disease	
Method	WB	IH	WB	IH	WB	IH	WB	IH
Arikawa et al [1] (n = 51)	15/15: N	15/15: N			29/36: N 5/36: A_1 2/36: A_2	 34/36: A_1 2/36: A_2	–	–
Morandi et al [24] (n = 11)	7/11: N 4/11: A_1	7/11: N 4/11: A_1			– –	– –	– –	– –
Nomura et al [27] (n = 4)	– –	– –			– –	2/4: N 1/4: A_1 1/4: A_2	– –	– –
Patel et al [29] (n = 5)	5/5: N	–			–	–	–	–
Uchino et al [36] (n = 3)	3/3: N	–			–	–	–	–
Voit et al [37] (n = 1)	1/1: A_1	1/1: A_1			–	–	–	–
Our study (n = 14)	–	6/6: N	–	1/1: N	–	5/5: N	–	2/2: N

WB, Western blot analysis; IH, immunohistochemistry; N, normal content and/or distribution of dystrophin; A, abnormal; A_1, abnormality in WB or presence of fibers without dystrophin (and/or abnormally distributed dystrophin); A_2, absence of dystrophin.

Dystrophin, desmin and vimentin

The dystrophin distribution was normal in all types of CMD (Fig. 2). Immunohistochemical desmin distribution was essentially the same as in the controls. Vimentin was present in the interstitial tissue in the patients and controls, but the muscle fibers were unstained.

Discussion

Histological characteristics were studied in all 30 patients. Our results with respect to morphological changes in muscle biopsies from pure-CMD, O-CMD, F-CMD and MEB-D patients indicated that there were no morphological hallmarks to differentiate these four CMD types. Very few reports are available in the literature, especially on MEB-D, probably because, initially, muscular dystrophy was overshadowed by severe cerebral and ocular malformations [9, 19]. Similar localization of MEB-D changes might be found in mitochondrial encephalomyopathy. However, we did not have any indication for the latter because increase of lipid and presence of ragged-red fibers were not observed in the biopsies. Furthermore, lactate and pyruvate levels were normal in both serum and CSF. Reports have indicated that muscle changes in CMD do not differ from those observed in patients reported to have O-CMD, F-CMD, or MEB-D [9, 14, 19, 34].

It is known that large numbers of hyaline fibers and groups of basophilic fibers are indicative of Duchenne muscular dystrophy, while ring fibers, fiber splitting and internal nuclei may indicate limb girdle dystrophy. Although these pathological characteristics are indicative of a particular type of dystrophy, they are not pathognomonic. In CMD, these pathognomonic characteristics are also lacking. Although the pattern was clearly dystrophic (fibrosis, diameter variability, and rounded muscle fibers), there was great variability between the parameters studied in each group. Even per patient, this histological variability was often considerable: the first biopsy of patient no. 18 did not show many fat cells, but in the second and third biopsies, the fat cell infiltration was extensive. It should be realized that the dystrophic characteristics in muscle biopsy specimens of all types of CMD vary considerably and generally depend on the stage of the disorder and are usually progressive. In fact, no significant differences were found between the groups. However, fat cell infiltration was found to increase with increasing age in pure-CMD. Owing to the lack of biopsies from patients in the higher age categories and the small number of patients with

O-CMD, F-CMD and MEB-D in our study, age-related conclusions could not be drawn from the data on these groups. Finally, no relation was found between muscle pathology and the clinical severity of the disease.

In 14 patients, comprising 6 pure-CMD, 1 O-CMD, 5 F-CMD and 2 MEB-D, immunohistochemistry with dystrophin antibody staining could be performed. In the literature only a few reports are available on dystrophin in CMD with or without involvement of the central nervous system [1, 24, 29, 36, 37]. The data from the literature and our data are summarized in Table 3. In contrast to some of the references in this table, the distribution pattern of dystrophin in all of our cases was normal. Although our patients were carefully matched with controls, it might be possible that our patients with a normal dystrophin distribution contained lower quantities of this molecule or that the type of dystrophin (smaller or larger molecules) was different from normal. Western blot analysis has been proposed to resolve this problem. However, in a recent study [37] the results of Western blot analysis were found to be largely compatible with those from immunofluorescence. It is doubtful whether some of the publications in Table 3 represent typical CMD [1, 27]. CK values were not always mentioned [15, 26] and DNA-gen analysis was not performed in most cases. Therefore, more atypical cases or clinical variants of other dystrophies may have been present in these cases. Our data indicate a more homogeneous population: CK values were normal or only slightly increased and the findings in muscle biopsies were different from those generally found in Duchenne or Becker muscular dystrophy [3, 5, 6, 15, 16, 23, 25, 26].

Recently [21], a greatly reduced staining for dystrophin-associated proteins was found for F-CMD patients, but not for non-F-CMD patients. Both groups showed normal or almost normal dystrophin distributions, which is in agreement with our results.

In conclusion, our study on morphological parameters of muscle biopsies from 30 patients with congenital muscular dystrophy, 8 with involvement of the central nervous system, 5 with involvement of both the brain and the eyes and 2 occidental types, did not reveal any differences between the subdivided groups except for a statistically significant higher amount of fat in the pure-CMD patients who were older than 2 years of age. Moreover, the cytoskeletal proteins vimentin and desmin showed a normal distribution in all of the patients examined, including O-CMD, F-CMD, and MEB-D.

References

1. Arikawa E, Ishihara T, Arahata K, Nonaka I, Sugita H (1991) Immunocytochemical analysis of dystrophin in congenital muscular dystrophy. J Neurol Sci 105:79-87
2. Banker BQ (1986) Congenital muscular dystrophy. In: Engel AG, Banker BQ (eds) Myology, vol 2. McGraw-Hill, New York, pp 1367-1382
3. Bonilla E, Samitt CE, Miranda AF, Hays AP, Salviati G, DiMauro S, Kunkel LM, Hoffman EP, Rowland LP (1988) Duchenne muscular dystrophy: deficiency of dystrophin at the muscle cell surface. Cell 54:447-452
4. Bordarier C, Aicardi J, Goutieres F (1984) Congenital hydrocephalus and eye abnormalities with severe developmental brain defects: Warburg's syndrome. Ann Neurol 16:60-65
5. Burghes AHM, Logan C, Hu X, Belfall B, Worton RG, Ray PN (1987) A cDNA clone from the Duchenne/Becker muscular dystrophy gene. Nature 328:434-437
6. Chelly J, Gilgenkrantz H, Lambert M, Hamard G, Chafey P, Récan D, Katz P, de la Chapelle A, Koenig M, Ginjaar IB, Fardeau M, Tomé F, Kahn A, Kaplan J-C (1990) Effect of dystrophin gene deletions on mRNA levels and processing in Duchenne and Becker muscular dystrophies. Cell 63:1239-1248
7. Dambska M, Wisniewski K, Sher J, Solish G (1982) Cerebro-oculo-muscular syndrome: a variant of Fukuyama congenital cerebromuscular dystrophy. Clin Neuropathol 1:93-98
8. Dobyns WB, Kirkpatrick JB, Hittner HM, Roberts RM, Kretzer FL (1985) Syndromes with lissencephaly II, Walker-Warburg and cerebro-oculo-muscular syndromes and a new syndrome with type II lissencephaly. Am J Med Genet 22:157-195
9. Dobyns WB, Pagon RA, Armstrong D, Curry CJR, Greenberg F, Grix A, Holmes LB, Laxova R, Michels VV, Robinow M, Zimmerman RL (1989) Diagnostic criteria for Walker-Warburg syndrome. Am J Med Genet 32:195-210
10. Dobyns WB, Pagon RA, Curry CJR, Greenberg F (1990) Regarding Walker-Warburg syndrome and muscle-eye-brain disease (letter to the editor). Am J Med Genet 36:373-374
11. Donner M, Rapola J, Somer H (1975) Congenital muscular dystrophy: a clinico-pathological and follow-up study of 15 patients. Neuropädiatrie 6:239-258

12 Echenne B, Arthuis M, Billard C, Campos-Castello J, Castel Y, Dulac O, Fontan D, Gauthier A, Kulakowski S, De Meuron G, Moore JR, Nieto-Barrera M, Pages M, Param D, Pavone L, Ponsot G (1986) Congenital muscular dystrophy and cerebral CT scan anomalies results of a collaborative study of the Société de Neurologie Infantile J Neurol Sci 75 7-22
13 Fukuyama Y, Kawazura M, Haruna H (1960) A peculiar form of congenital progressive muscular dystrophy report of fifteen cases Pediatr Univ Tokyo (Tokyo) 4 5-8
14 Fukuyama Y, Osawa M, Suzuki H (1981) Congenital progressive muscular dystrophy of the Fukuyama type clinical, genetic and pathological considerations Brain Dev 3 1-29
15 Hoffman EP, Monaco AP, Feener CA, Kunkel LM (1987) Conservation of the Duchenne muscular dystrophy gene in mice and humans Science 238 347-350
16 Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM (1987) Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals Cell 50 509-517
17 Krijgsman JB, Barth PG, Stam FG, Slooff JL, Jaspar HHJ (1980) Congenital muscular dystrophy and cerebral dysgenesis in a Dutch family Neuropadiatrie 11 108-120
18 Leyten QH, Gabreels FJM, Joosten EMG, Renier WO, Ter Laak HJ, Ter Haar BGA, Stadhouders AM (1986) An autosomal dominant type of congenital muscular dystrophy Brain Dev 8 533-537
19 Leyten QH, Gabreels FJM, Renier WO, Ter Laak HJ, Sengers RCA, Mullaart RA (1989) Congenital muscular dystrophy J Pediatr 115 214-221
20 Leyten QH, Renkawek K, Renier WO, Gabreels FJM, Mooy CM, Ter Laak HJ, Mullaart RA (1991) Neuropathological findings in muscle-eye-brain disease (MEB-D) neuropathological delineation of MEB-D from congenital muscular dystrophy of the Fukuyama type Acta Neuropathol 83 55-60
21 Matsumura K, Nonaka I, Campbell KP (1993) Abnormal expression of dystrophin-associated proteins in Fukuyama-type congenital muscular dystrophy Lancet 341 521-522
22 McMenamin JB, Becker LE, Murphy EG (1982) Congenital muscular dystrophy a clinicopathologic report of 24 cases J Pediatr 100 692-697
23 Monaco AP, Neve RL, Colletti-Feener C, Bertelson CJ, Kurnit DM, Kunkel LM (1986) Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene Nature 323 646-650

24 Morandi L, Gussoni E, Mora M, Gebbia M, Merlini L, Cornelio F (1990) Dystrophin analysis in congenital muscular dystrophies (abstract) J Neurol Sci 98 [Suppl] 436
25 Morandi L, Mora M, Gussoni E, Tedeschi S, Cornelio F (1990) Dystrophin analysis in Duchenne and Becker muscular dystrophy carriers correlation with intracellular calcium and albumin Ann Neurol 28 674-679
26 Nicholson LVB, Johnson MA, Gardner-Hedwin D, Bhattacharya S, Harris JB (1990) Heterogeneity of dystrophin expression in patients with Duchenne and Becker muscular dystrophy Acta Neuropathol 80 239-250
27 Nomura Y, Ehara M, Yamanaka T, Arahata K, Nonaka I, Segawa M (1990) Heterogeneity of congenital muscular dystrophy (abstract) J Neurol Sci 98 [Suppl] 437
28 Pagon RA, Clarren SK, Milam DF Jr, Hendrickson AE (1983) Autosomal recessive eye and brain anomalies Warburg syndrome J Pediatr 102 542-546
29 Patel K, Voit T, Dunn MJ, Strong PN, Dubowitz V (1988) Dystrophin and nebulin in the muscular dystrophies J Neurol Sci 87 315-326
30 Raitta G, Lamminen M, Santavuori P, Leisti J (1978) Ophthalmological findings in a new syndrome with muscle, eye and brain involvement Acta Ophthalmol (Copenh) 56 465-472
31 Santavuori P, Leisti J, Kruus S (1977) Muscle, eye and brain disease a new syndrome (abstract) Neuropadiatrie 8 [Suppl] 553
32 Santavuori P, Somer H, Sainio K, Rapola J, Kruus S, Nikitin T, Ketonen L, Leisti J (1989) Muscle-eye-brain disease (MEB) Brain Dev 11 147-153
33 Santavuori P, Pihko H, Sainio K, Lappi M, Somer H, Haltia M, Raitta C, Ketonen L, Leisti J (1990) Muscle-eye-brain disease and Walker-Warburg syndrome (letter to the editor) Am J Med Genet 36 371-372
34 Topaloğlu H, Yalaz K, Renda Y, Çağlar M, Goğus S, Kale G, Gucuyener K, Nurlu G (1991) Occidental type cerebromuscular dystrophy a report of eleven cases J Neurol Neurosurg Psychiatry 54 226-229
35 Towfighi J, Sassani JW, Suzuki K, Ladda RL (1984) Cerebro-ocular dysplasia-muscular dystrophy (COD-MD) syndrome Acta Neuropathol (Berl) 65 110-123
36 Uchino M, Araki S, Teruhisa M, Teramoto H, Nakamura T, Yasutake T (1989) Localization and characterization of dystrophin in muscle biopsy specimens from Duchenne muscular dystrophy and various neuromuscular disorders Muscle Nerve 12 1009-1016

37. Voit T, Stuettgen P, Cremer M, Goebel HH (1991) Dystrophin as a diagnostic marker in Duchenne and Becker muscular dystrophy: correlation of immunofluorescence and Western blot. Neuropediatrics 22:152-162
38. Warburg M (1978) Hydrocephaly, congenital retinal nonattachment and congenital falciform fold. Am J Ophthalmol 85:88-94

Neuropathological findings in muscle-eye-brain disease (MEB-D)

Neuropathological delineation of MEB-D from congenital muscular dystrophy of the Fukuyama type

Q.H. Leyten, K. Renkawek, W.O. Renier, F.J.M. Gabreëls,
C.M. Mooy, H.J. ter Laak, R.A. Mullaart

Acta Neuropathologica 1991;83:55-60

Abstract

Congenital muscular dystrophy (CMD) associated with cerebro-ocular dysplasia named muscle-eye-brain disease (MEB-D) is described in two sisters. Progressive hypotonia, mental retardation and severe visual failure appeared immediately after birth. Pathological examination demonstrated muscular dystrophy, hydrocephalus, type II lissencephaly and defective eye development of foetal origin. The great similarity of the clinical and neuropathological picture of both sisters is in agreement with an autosomal recessive inheritance. Neuropathological distinction between Fukuyama-CMD and MEB-D is a more severe and earlier cerebral developmental defect and the association with ocular dysplasia in MEB-D.

Congenital muscular dystrophy (CMD) appears to be a heterogeneous group of disorders. CMD can be the only pathology, but can also be associated with cerebral abnormalities (Fukuyama-CMD [7]) or with cerebral and ocular dysgenetic changes (muscle-eye-brain disease, MEB-D [12, 14] or congenital cerebro-ocular-muscle dysgenesis, COD-MD [6, 8, 18]). MEB-D and COD-MD have to be considered identical [4]. In the majority of the cases of Fukuyama-CMD and MEB-D brain abnormalities consist of hydrocephalus, lissencephaly, polymicrogyria and gliomesodermal proliferation. In MEB-D, ophthalmic malformations are associated, such as immature anterior chamber angle, cataract, retinal dysplasia, retinal detachment, abnormal retinal pigment epithelium and microphthalmia, persistent hyperplastic primary vitreous (PHPV) and hypoplasia of the optic nerve [7, 14, 16, 18].

On clinical grounds, Fukuyama-CMD can be differentiated from MEB-D [14]. Concerning the pathological aspects, it remains an unresolved problem whether they are separate entities or different expressions of the same disease. Brain anomalies are present in both instances, but eye changes are rarely found in Fukuyama-CMD [2, 19].

In this article we describe the clinical and neuropathological findings in two sisters with MEB-D. The results are compared with neuropathological data in the literature concerning MEB-D and Fukuyama-CMD, and the differences are discussed.

Clinical and pathological findings

Case 1

A girl, the first child of healthy, non-consanguineous parents was born after an uneventful pregnancy of 37 weeks. Postpartum the child did not breath spontaneously and artificial respiration was used. From birth generalised hypotonia and head lag were present. Deep tendon reflexes were absent. There was a macrocephaly (occipito-frontal circumference (OFC) $P > 97.5$) with frontal bossing. The girl appeared profoundly retarded, reacting to auditory and tactile stimuli but not to visual ones. Ophthalmological examination revealed microphthalmia and blepharophimosis of the right eye, clouding of the cornea, choroid atrophy and retinal detachment of the left eye. On the 2nd day after birth hemiconvulsions occurred, which were treated successfully with phenobarbital and diphenylhydantoin. At the age of 2 months the girl suddenly died. A brother of the mother had a child with microphthalmia of the right eye.

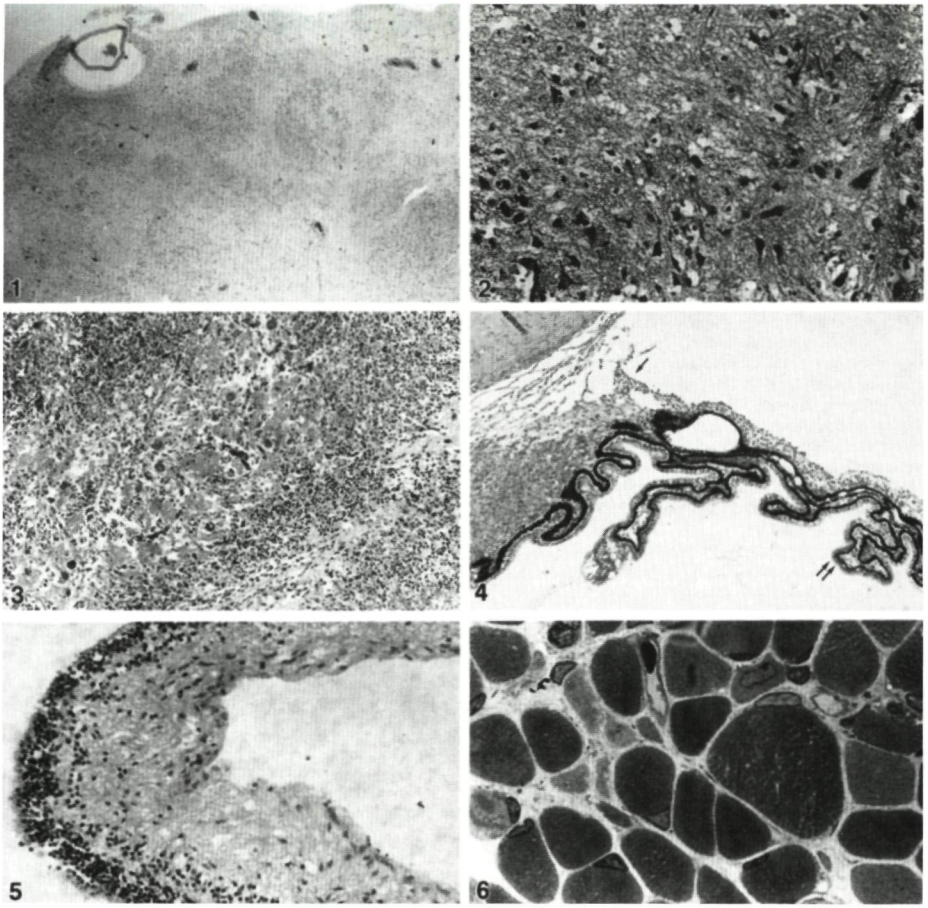

Fig. 1. Cerebral cortex, complete lack of lamination. Clustered neurons, hazardly distributed in the cortical ribbon. H&E, x 20.

Fig. 2. Spatial disarray and abnormal position of the neurons. Klüver-Barrera, x 100.

Fig. 3. Completely distorted architecture of the cerebellum; the various layers are intermingled inextricably. H&E, x 50.

Fig. 4. Section of left eye demonstrating in the chamber angle anterior insertion of the iris root on trabecular meshwork (↓) and elongated ciliary processes with attachment to the posterior iris (↓↓). H&E, x 50.

Fig. 5. Non-attached retina with atrophy of the photoreceptor layer and glial proliferation of the inner retina. H&E, x 100.

Fig. 6. Muscle section from the quadriceps biopsy. Note the rounded fibres and increased diameter variability. Toluidine blue, Epon, x 500.

Biochemical investigations of serum and urine gave normal values except from increased creatine kinase (CK: 1130 U/l, normal <90 U/l) and glutamate-oxytransferase (GOT: 70 U/l, normal <40 U/l). Cerebrospinal fluid (CSF) had increased protein content (2400 mg/l). Microbiological examination excluded infections such as toxoplasma, cytomegalovirus, herpes or rubella virus. Chromosomal examination revealed a normal pattern (46 XX). Muscle biopsy was refused. EEG showed a normal background pattern but with spike and wave paroxysms over the right hemisphere. EMG revealed an increased number of brief polyphasic potentials with small amplitude. Echo scan of the brain was normal.

Pathological examination. General autopsy revealed small follicle cysts in the right ovarium. In the adrenals some nodular hyperplasia occurred. Histological examination of the skin excluded ectodermal dysplasia.

Neuropathological examination. The brain weighted 410 g, was fixed in 10% formalin, embedded in paraffin and sections were stained with H&E, Klüver-Barrera, Bodian and Holzer methods.

Gross examination of the brain revealed a type II lissencephaly. Only a few poorly marked grooves were seen in the frontal lobes. The meninges were thickened. The olfactory bulbs, optic tracts and chiasma were missing. The cerebellum was hypoplastic, with absence of the vermis. Cerebellar surface was smooth with poorly developed foliae. Above the cerebellum a thin wall cyst expanding out of the fourth ventricle through the midline of the cerebellum was found, corresponding to Dandy-Walker malformation. The brain stem and spinal cord appeared normal.

Coronal sections showed lacking septum pellucidum, a very thin corpus callosum and enormously dilated ventricular system. The border between white and grey matter was obliterated, the basal ganglia and thalami were present. In both cerebellar hemispheres dentate nucleus was visible.

Microscopical examination revealed complete absence of cerebral cortex lamination (Fig. 1). Neurons of various size were either scattered or clustered into glomerular or columnar formations at different cortical depths. Spatial disarray and abnormal position of the neurons were seen (Fig. 2). Multiple heterotopias were visible under the ependyma of both lateral ventricles. In the cortex diffuse spongiosis and gliomesodermal proliferation in the superficial cortical area was evident. The meninges showed an abundant number of vessels and marked proliferation of the mesenchymal meshwork. Myelinated fibres were visible only in the subcortical areas.

The architecture of the cerebellum was markedly destroyed. The foliae were fused. Neither granular layer nor Purkinje cells layer were normally formed. Dispersed islands of accumulated granular cells with admission of Purkinje cells or clusters of molecular layer incorporated into granular cells were seen (Fig. 3). Heterotopic foci of neurons in the white matter were noted.

The basal ganglia, hippocampus, brain stem and spinal cord were unremarkable. The vessels of the meninges and the brain exhibited advanced proliferative changes of their walls.

Examination of the eyes. Both eyes were microphthalmic. In the left eye fetal configuration of the chamber angle and anterior polar cataract was noted. In addition elongated fused ciliary processes with attachment to the posterior iris were present (Fig. 4). The retina was detached with atrophy of the photoreceptor layer and glial proliferation of the inner retina. Incomplete retinal vascularisation and irregular pigmentation of the retinal pigment epithelium were present (Fig. 5). Neuroectodermal derivates, like the sphincter and dilator pupillary muscle were poorly developed; the iris pigment epithelium was double layered and irregularly pigmented. The right eye revealed a vascularised corneal stroma, breaks in Descemet's layer and loss of endothelium, agenesis of trabecular meshwork, iris coloboma and partly hypoplastic ciliary body. In addition, agenesis of iris pigment epithelium and ciliary body epithelium, agenesis of lens, vitreous, retina, choroid and optic nerve were found.

Case 2

The youngest sister of patient 1, was born after an uneventful pregnancy terminated by vacuum extraction. At birth generalised hypotonia was present. Deep tendon reflexes were absent. Contractures were lacking. There was a macrocephaly (OFC P > 97.5) with frontal bossing. The girl appeared profoundly retarded, reacting to auditory and tactile stimuli but not to visual ones. Ophthalmological examination revealed microphthalmia and blepharophimosis of the right eye, clouding of the cornea and fetal anterior chamber angle of the left eye. Cerebral CT scanning showed agyria and a large cyst above the cerebellum with aplasia of the inferior vermis and supratentorial hydrocephalus (Dandy-Walker variant). Increased intracranial pressure required ventriculoperitoneal drainage, and at the age of 4 months the girl died because of purulent meningitis caused by *Klebsiella pneumoniae* and *Escherichia coli.*

Biochemical investigation of serum revealed an increased CK activity (973 and 1540 U/l). GOT, calcium, phosphate, myoglobulin, lactate and pyruvate as well as fatty acids, dicarbon acid and myoglobin were normal. CSF protein

content was elevated (1576 mg/l). Microbiological examination excluded viral infection. Chromosomal examination revealed a normal pattern (46 XX). EEG showed a diffuse slowing of the background pattern without epileptic discharges. EMG revealed increased number of brief polyphasic potentials with small amplitude. Motor nerve conduction velocity was normal.

Muscle biopsy. A muscle biopsy from the quadriceps was performed at the age of 2 months and revealed rounded muscle fibres and clearly increased diameter variability (Fig. 6). There was a moderate lipomatosis and a moderate endomysial and perimysial fibrosis. Fibres with internal nuclei were scarce. Opaque fibres were sometimes found and atrophic fibres often showed hyalinisation. One relatively large fibre filled with some swollen nuclei with clearly visible nucleoli was also observed.

The above-mentioned findings correspond to muscular dystrophy.

Pathological examination. Autopsy of the body revealed slight splenomegaly of the weight (250 g, normal 160 g) and infectious changes in the spleen.

Neuropathological examination. Brain, weighting 770 g, was fixed in 10% formalin. Brain surface was devoid of gyri and sulci (lissencephaly). The meninges were severely thickened and massive purulent exsudate was present. The olfactory tracts and optic chiasma were absent. The cerebellum was hypoplastic, the vermis was residual. The brain stem and spinal cord appeared normal. Above the cerebellar surface a large cyst with a wall of about 3-mm thickness filled with purulent mass was found. Supratentorial hydrocephalus and enormously dilated ventricle system were present.

Microscopical examination showed similar changes as have been described in the first child. Severely disorganised cortical lamination without horizontal pattern was seen. Massive gliomesodermal proliferation was present. The neurons were randomly distributed, some were placed in a rotated position. The vessels showed enormous proliferation of their walls. In the meninges, in the cortex and white matter the vessels were surrounded by massive monocyte infiltrations. In the white matter of the parietal lobe dispersed foci of necrosis were present.

In the white matter only single fibres were myelinated. In the basal ganglia, hippocampus, brain stem and spinal cord, the intensity of the inflammation was less pronounced and white and grey substance were well developed.

The cerebellar structure was severely abnormal. No developed foliae or cortical layers were recognisable. Most of the cerebellar cortex was composed of granular neurons which formed small groups of cells lying between hazardly mounted Purkinje cells. In the vicinity of the fourth ventricle proliferation of the

Table 1. Brain, eye and muscle findings in muscle-eye-brain disease (MEB-D) cases

	Clinical data	Brain
1 Williams et al. [21]	Hypotonia, 3 cases, 2 siblings	Hydrocephalus, occipital encephalocele, agenesis of olfactory and optic tract, corpus callosum, hypoplasia of vermis and cerebellum, lissencephaly, cerebellar micropolygyria, gliomesodermal proliferation, heterotopia
2 Towfighi et al [18]	Hypotonia, 7 cases in 4 families	Hydrocephalus, cysts, hypoplasia of olfactory and optic tract, vermis and brain stem, lissencephaly, micropolygyria, gliomesodermal proliferation, heterotopia
3 Heggie et al [8]	Hypotonia, 2 siblings	Hydrocephalus, meningoencephalocele, hypoplasia of vermis and brain stem, lissencephaly, cortical dysplasia, heterotopia
4 Pavone et al [11]	Hypotonia, 3 cases in 1 family	Hydrocephalus, lissencephaly, arhinencephaly, cerebral and cerebellar agyria, pachygyria and/or micropolygyria, heterotopia, hypoplasia of pyramidal tracts and cerebellum, Dandy-Walker cyst
5 Federico et al. [6]	Hypotonia, 2 cases	Hydrocephalus, lissencephaly, agenesis of corpus callosum, optic nerve, chiasma and olfactory bulb and lobe, aplasia of pyramides, spongiotic white matter, heterotopia, absence of cerebellar vermis
6 Dambska et al. [3]	Hypotonia, 3 siblings	Hydrocephalus, lissencephaly, agenesis of the olfactory bulbs, corpus callosum and of anterior commissure, hypoplasia of optic nerves, fossa posterior cyst, absence of the cerebellar vermis, cerebral and cerebellar micropolygyria with cortical disorganisation
7 Chijiiwa et al [2]	Hypotonia, 2 cases	No histology, only clinical findings, i e. mental retardation and neuroradiological findings (CT), i.e. atrophy of the white matter and hydrocephalus
8. Our case	Hypotonia, mental retardation, 2 siblings	Hydrocephalus, Dandy-Walker cyst, agenesis of olfactory and optic tracts, septum, corpus callosum and vermis, lissencephaly without cortical lamination, heterotopia

PHPV Persistent hyperplastic primary vitreous; RPE: retinal pigment epithelium.

Eye	Muscle
Microphthalmia, retinal dysplasia, anterior chamber malformations, cataract	Variability in fibre size, fibrosis, focal necrosis with regeneration (found in 1 case)
Microphthalmia, retinal detachment and dysplasia, fetal chamber angle, cataract, PHPV, abnormal RPE, abnormal vascularisation and hypoplasia of the optic nerve	Variability in fibre size, endomysial fibrosis, basophilic fibres, necrosis
Microphthalmia, retinal detachment and dysplasia, cataract, immature anterior chamber angle, PHPV, optic nerve hypoplasia and coloboma	Variability in fibre size, adipose and fibrotic degeneration
No histology, only clinical findings, i e. shortened anterior chamber, fixed pupils and corticonuclear opacities in the lens	Severe degeneration of muscle fibres, fibrosis, lipomatosis
No histology, only clinical findings, i e microphthalmia, corneal opacities, Peter's anomaly, central leukoma, RPE abnormalities, malformation of optic disc	Variability in fibre size, fibrosis, central nuclei
Mesodermal dysgenesis of the anterior chamber of the left eye, Peter's anomaly	Severe intrafascicular fibrosis, necrotic fibres, foci of regeneration, variability in fibre size, swollen hyalinised fibres
No histology, only clinical findings and fluorescein angiography, high myopia with astigmatism, entropion of lower lids, optic nerve palor, capillary nonperfusion, dilated and irregular capillaries	Random variation in fibre size, marked decrease in the amount of muscle fibre
Microphthalmia, retinal dysplasia, fetal chamber, coloboma, cataract	Variability in fibre size, round fibres, basophilic fibres, adipose and fibrotic degeneration

ependyma and inflammatory process, growing above ependymal layer, were seen.

Discussion

The simultaneous occurrence of muscle, eye and brain developmental defects (MEB-D) is a rare combination. Cases with complete pathological description have infrequently been documented (Table 1). Similar cerebral neuropathological features in different cases suggest an identical clinico-pathological entity [3]. MEB-D can be considered as part of a larger spectrum of CMD. Other entities of the spectrum are the pure form of CMD and a Fukuyama type of CMD. Pathological criteria of the brain, eye and muscle changes can have additional diagnostic value in the classification of these entities.

The muscle pathology represents changes commonly described in the three entities of CMD [5]. The neuropathological features as has been described earlier in the Walker-Warburg syndrome (WWS) [20], such as congenital hydrocephalus, agyria, absence of cortical lamination, congenital retinal dysplasia, retinal detachment, and abnormalities of eye chambers [1], are also found in our cases. The WWS is now to be considered identical to MEB-D [4, 9, 15], although in WWS muscular dystrophy is not described in detail. Other neuropathological features as agenesis of optic chiasma, olfactory bulb, corpus callosum, dysplasia of the cerebellum, agenesis of vermis or cystic degeneration have been found in both MEB-D and in WWS. In the particular cases of Pavone et al. [11], Dambska et al. [3], Chijiiwa et al. [2] and Federico et al. [6], pathological brain and muscle findings similar to our cases have been detected, but ocular pathology was restricted to clinical ophthalmological examination. Also the two siblings described by Heggie et al. [8] as cerebro-ocular dysplasia-muscular dystrophy syndrome do not differ essentially from our cases.

The neuromorphological malformations in MEB-D are different from those described in Fukuyama-CMD. Most Fukuyama-CMD patients are microcephalic with hypomyelination of the white matter, have no or mild ventricle dilatation, no encephalocele and only exceptionally an ocular involvement. Polymicrogyria is frequently present in Fukuyama-CMD, while type II lissencephaly is prevalent in MEB-D [2]. Thus, MEB-D could be considered a more severe neurodevelopmental disturbance than Fukuyama-CMD.

Several aetiologies are possible. In Fukuyama-CMD, brain pathology can appear at different stages of development and even can precede muscle dystro-

phic changes [17]. A viral infection, probably of low virulence has been suggested as the cause of the proliferative, sclerosing process of the meninges, eventually leading to an internal hydrocephalus [21]. Eye damage might be explained by a local trauma early in the embryogenesis, or to local failure of the neural crest to develop a primary optic vesicle. As a unifying hypothesis explaining the malformation, a form of "neurocristopathy" has been suggested [13]. A deficiency in the number of crest cells, abnormal differentiation or a disturbance in the migration of the neural crest cells, may play a role in the malformation of the eyes [10]. Abnormalities in the neural crest may have occurred at any stage from their initial appearance to their final differentiation. This neural crest hypothesis does not rule out the theory of an intrauterine infection.

However, the high incidence of this disorder among siblings suggests a genetic determination. All known reports of MEB-D have an autosomal recessive inheritance [1-3, 5-8, 11, 14, 16, 18, 19, 21]. Because interfamilial differences are more pronounced than intrafamilial, genetic heterogeneity and the existence of allelic mutations within MEB-D is possible. This aetiological heterogeneity might be caused by allelic mutations in different genes.

Our cases of two siblings being similarly affected imply a genetic influence. In the same family another child had microphthalmia, but normal intelligence. It is tempting to hypothesise that the genetic factor in the family has a variable expression.

In conclusion, our study suggests that in Fukuyama-CMD and in MEB-D, although the muscular dystrophy pattern seems comparable, the neuropathological data are clearly different. MEB-D is a more severe developmental disturbance than Fukuyama-CMD. The genetic influences seem to have their expression at different stages of the neural crest development.

References

1. Bordarier C, Aicardi J, Goutieres F (1984) Congenital hydrocephalus and eye abnormalities with severe developmental brain defects: Warburg's syndrome. Ann Neurol 16:60-65
2. ChijiiwaT, Nishimura M, Inomata H, Yamana T, Narazaki O, Kurokawa T (1983) Ocular manifestations of congenital muscular dystrophy (Fukuyama type). Ann Ophthalmol 15:921-928
3. Dambska M, Wisniewski K, Sher J, Solish G (1982) Cerebro-oculo-muscular syndrome: a variant of Fukuyama congenital cerebromuscular dystrophy. Clin Neuropathol 1:93-98
4. Dobyns WB, Pagon RA, Curry CJR, Greenberg F (1990) Letter to the Editor: Response to Santavuori et al. Regarding Walker-Warburg syndrome and muscle-eye-brain disease. Am J Med Genet 36:373-374
5. Donner M, Rapola J, Somer H (1975) Congenital muscular dystrophy. A clinico-pathological and follow-up study of 15 patients. Neuropädiatrie 6:239-258
6. Federico A, Dotti MT, Malandrini A, Guazzi GC, Hayek G, Simonati H, Rizzuto N, Toti P (1988) Cerebro-ocular dysplasia and muscular dystrophy: report of two cases. Neuropediatrics 19:109-112
7. Fukuyama Y, Osawa M, Suzuki H (1981) Congenital progressive muscular dystrophy of the Fukuyama type: clinical, genetic and pathological considerations. Brain Dev 3:1-29
8. Heggie P, Grossniklaus HE, Roessmann U, Chou SM, Cruse RP (1987) Cerebro-ocular dysplasia-muscular dystrophy syndrome: report of two cases. Arch Ophthalmol 105:520-524
9. Leyten QH, Gabreëls FJM, Renier WO, ter Laak HJ, Sengers RCA, Mullaart RA (1989) Congenital muscular dystrophy. J Pediatr 115:214-221
10. Mooy CM, Clark BJ, Lee WR (1990) Posterior axial corneal malformation and uveoretinal angiodysgenesis: a neurocristopathy? Graefes Arch Clin Exp Ophthalmol 228:9-18
11. Pavone L, Gullotta F, Grasso S,Vannucchi C (1986) Hydrocephalus, lissencephaly, ocular abnormalities and congenital muscular dystrophy. A Warburg syndrome variant? Neuropediatrics 17:206-211
12. Raitta C, Lamminen M, Santavuori P, Leisti J (1978) Ophthalmological findings in a new syndrome with muscle, eye and brain involvement. Acta Ophthalmol (Copenh) 56:465-472

13. Rhodes RH, Carbajal UM (1982) Ocular malformation involving tissue of neural crest derivation. Graefes Arch Clin Exp Ophthalmol 219:135-139
14. Santavuori P, Somer H, Sainio K, Rapola J, Kruus S, Nikitin T, Ketonen L, Leisti J (1989) Muscle-eye-brain disease (MEB). Brain Dev 11:147-153
15. Santavuori P, Somer H, Pihko H, et al. (1990) Letter to the Editor: Muscle-eye-brain disease and Walker-Warburg syndrome. Am J Med Genet 36: 371-372
16. Sasaki M, Yoshioka K, Yanagisawa T, Nemoto A, Takasago Y, Nagano T (1989) Lissencephaly with congenital muscular dystrophy and ocular abnormalities: cerebro-oculo-muscular syndrome. Childs Nerv Syst 5:35-37
17. Takada K, Nakamura H, Suzumori K, Ischikawa T, Sugiyuma N (1987) Cortical dysplasia in a 23-weeks fetus with Fukuyama congenital muscular dystrophy (FCMD). Acta Neuropathol (Berl) 74:300-306
18. Towfighi J, Sassani JW, Suzuki K, Ladda RL (1984) Cerebro-ocular dysplasia-muscular dystrophy (COD-MD) syndrome. Acta Neuropathol (Berl) 65:110-123
19. Tsutsumi A, Uchida Y, Osawa M, Fukuyama Y (1989) Ocular findings in Fukuyama-type congenital muscular dystrophy. Brain Dev 11:413-419
20. Warburg M (1978) Hydrocephaly, congenital retinal nonattachment, and congenital calciform fold. Am J Ophthalmol 85:88-94
21. Williams RS, Swisher CN, Jennings M, Ambler M, Caviness VS Jr (1984) Cerebro-ocular dysgenesis (Walker-Warburg syndrome). Neurology 34: 1531-1541

Abnormal merosin (or laminin-α_2) expression in congenital muscular dystrophies

An immunohistochemical study

H.J. ter Laak, Q.H. Leyten, F.J.M. Gabreëls,
H. Kuppen, W.O. Renier, R.C.A. Sengers

Submitted for publication

Abstract

Muscle biopsies of 13 congenital muscular dystrophy (CMD) patients were investigated for the presence of merosin (the heavy chain protein of laminin M or in new nomenclature laminin-α_2). Merosin levels were normal in 6 out of the 8 patients with pure-CMD, in the 3 patients considered to have Fukuyama-CMD (F-CMD) and in the 2 patients with the Walker-Warburg syndrome (WWS). The 2 pure-CMD patients with white matter hypodensity showed severely decreased merosin expression and contrary to the literature on Japanese F-CMD, we did not find any marked deficiency of merosin in our F-CMD. As the merosin gene and the gene for Japanese F-CMD are located on different chromosomes (6 and 9, respectively), merosin deficiency in Japanese F-CMD is probably secondary to another as yet unknown defect.

According to two European Neuromuscular Centre (ENMC) workshops [5, 6], the congenital muscular dystrophies (CMDs) can be classified into classical or pure-CMD with only muscle involvement, Fukuyama type CMD (F-CMD) with both muscle and structural brain abnormalities, and into CMD with muscle, eye and brain abnormalities or muscle-eye-brain disease (MEB-D), including the Finnish type (F-MEB-D) and the Walker-Warburg syndrome (WWS), a more severe variant of MEB-D.

Muscle fibres are surrounded by a specialized extracellular matrix -the basement membrane (BM)- that contains laminin M, type IV collagen, fibronectin and heparin-sulphate proteoglycan. The BM plays a major role in muscle development and regeneration, in signal transmission, nerve adhesion and in the distribution and transmission of force. Laminin M is a cross-shaped protein with one large chain (the M-chain or merosin in postnatal muscle) and two smaller side chains (B1 and B2); these chains are encoded by chromosomes 6 [23], 7 [16] and 1 [14], respectively.

Besides laminin M, laminin A isoform may be discerned. Laminin A also contains one large chain (the A-chain, encoded by chromosome 18) and the two smaller chains, B1 and B2. In muscle, laminin A is found in the small blood vessels and in regenerating muscle fibres [7].

According to new nomenclature [3], the terms laminin A, laminin M, A-chain, M-chain or merosin, B1-chain and B2-chain have been replaced by the terms laminin-1, laminin-2, laminin-α_1, laminin-α_2, laminin-β_1 and laminin-γ_1, respectively.

The extracellular BM is linked to subsarcolemmal dystrophin via a transmembrane glycoprotein complex. This complex is composed of various tightly bound dystrophin-associated proteins (DAPs or DAGs) with different molecular weights (25, 35, 43, 50, 59 and 156 kDa). The 43 (transmembrane) and 156 (extracellular) kDa DAGs represent products of the same gene [9] located on chromosome 3 [10]. Laminin, especially merosin, is bound to 156 kDa DAG [9, 18] on the outer side of the sarcolemma. Apparently, an intact dystrophin-DAG/DAP-laminin complex is an essential condition for normal muscular function. Dystrophin absence and abnormalities cause X-linked recessive Duchenne dystrophy and Becker type dystrophy; absence of 50 kDa DAG or adhalin is found in severe childhood autosomal recessive muscular dystrophy or SCARMD [13].

Recently, 13 merosin-negative pure-CMD patients (out of 20) have been described [21]. With MRI, white matter abnormalities were seen in 8 merosin deficient pure-CMD patients (seven patients described by Mercuri et al. [15] and

Table 1. Signs and symptoms, genetic, laboratory, radiological and neurophysiological characteristics in congenital muscular dystrophy (CMD)

	Congenital muscular dystrophy (CMD)												
	Classical or pure-CMD								F-CMD			WWS	
Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Age at muscle biopsy (yr)	0	0	1	3	3	5	1	8	1	3	4	0	5
Sex	M	M	F	M	M	M	F	F	F	M	F	M	M
Consanguinity	–	–	+	–	–	–	–	–	+	–	+	–	–
Congenital hypotonia	+	+	+	+	+	+	+	+	+	+	+	+	+
Joint contractures at birth	+	–	+	–	–	–	–	+	–	+	+	+	+
Rapid progressive course (motor retardation)	–	–	–	–	–	+	–	–	–	–	–	–	–
DNA analysis	N	np	np	np	N	N	N	N	N	np	np	np	np
CNS involvement													
– CSF abnormalities	N	np	N	N	N	np	N	N	N	N	N	N	N
– EEG abnormalities	–	–	–	+	–	–	–	+	+	+	+	+	+
– Convulsions / epilepsy	–	–	–	–	–	–	–	–	+	+	+	+	+
– White matter hypodensity (CT/MRI)	–	–	–	–	–	–	+	+	+	+	+	+	+
– Encephalocele	–	–	–	–	–	–	–	–	–	–	–	+	–
– Intelligence *	N	N	N	N	N	N	N	N	–	–	–	–	–
Opthalmological disorder	–	–	–	–	–	–	–	–	–	–	–	+	+

F-CMD, Fukuyama CMD; WWS, Walker-Warburg syndrome; M, male; F, female; N, normal; np, not performed; –, absent; +, present; * normal intelligence: IQ > 70.

Table 2. Morphological parameters of muscle biopsy in congenital muscular dystrophy (CMD)

	Congenital muscular dystrophy (CMD)												
	Classical or pure-CMD								F-CMD			WWS	
Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Biopsy location	Q	Q	Q	RA	Q	Q	Q	Q	Sol	Q	Q	Q	Q
CK activity (normal < 90 U/l)	88	N	33	N	N	153	117	860	737	60	1192	1050	900
Fat	–	–	–	–	+	++	+	+	+	+++	–	–	+
Connective tissue	+	+	–	++	+	+	++	++	+	+++	+	+	+
Internal nuclei	–	–	–	–	–	–	+	+	–	++	–	–	–
Basophilic fibres	–	–	–	–	+	+	–	–	–	–	–	–	+
Necrotic fibres	–	–	–	–	+	–	+	–	–	–	–	–	+/–
Hyaline fibres	–	–	–	–	–	–	–	–	–	–	–	+/–	–
Percentage type I fibres	33	48	67	70	55	82	76	40	97	100	53	41	59
Type IIC fibres	+	–	–	–	+	++	+	–	–	NO	–	–	–
Atrophic fibres													
– type I	++	+++	++	+	+	+	+	+	+	++	–	+	+
– type II	++	+++	+	+	+++	+	–	+	+	NO	–	+	–
Hypertrophic fibres													
– type I	–	–	+	+	+++	–	++	++	–	+++	+	–	–
– type II	–	+++	+	+	+	–	++	++	–	NO	+	–	–
Immuno-expression													
– dystrophin	N	N	N	N	N	N	N	N	N	N	N	N	N
– desmin	N	N	N	N	N	N	N	N	N	N	N	N	N
– vimentin	N	N	N	N	N	N	N	N	N	N	N	N	N
– laminin	N	N	N	N	N	N	N	N	N	N	N	N	N
– merosin	N	N	N	N	N	N	D	D	N	N	N	N	N

F-CMD, Fukuyama-CMD; WWS, Walker-Warburg syndrome; N, normal, D, deficient, NO, not observed, Q, quadriceps muscle, Sol, soleus muscle, RA, rectus abdominis muscle; –, normal or absent, +/–, present, +, increased; ++, marked, +++, extensive

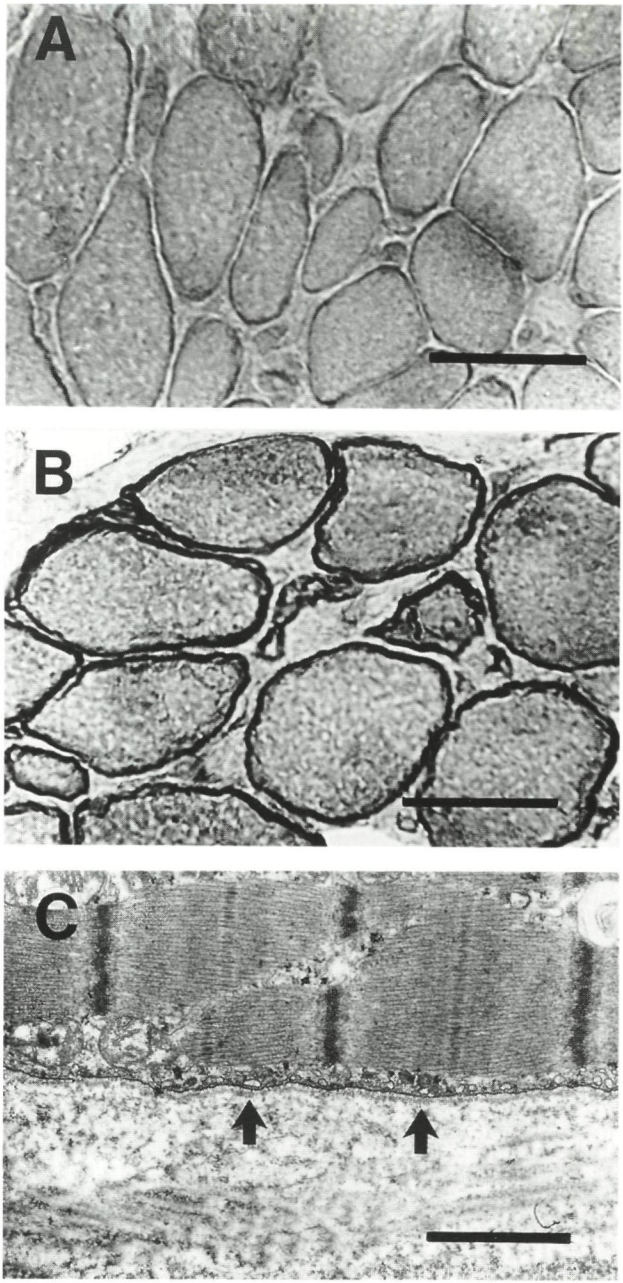

Fig 1. Quadriceps muscle. Decreased (A) and normal (B) merosin staining in "pure" CMD with white matter hypodensity (patient 7) and Fukuyama type CMD (patient 10). Normal basement membrane ultrastructure (arrows, C) in patient 7. Bars represent 50 μm (A and B) and 1 μm (C).

one by Voit et al. [22]). Furthermore, a severe reduction in merosin was observed in F-CMD [7].

In this study, we present and discuss our results concerning the immunodetection of laminin and merosin in 13 patients with various types of CMD.

Patients and Methods

The muscle biopsies of 13 patients were investigated; these 13 CMD patients comprised 8 pure-CMD patients (6 patients without and 2 with white matter hypodensities), 3 F-CMD patients and 2 patients with WWS. The clinical and morphological data are summarized in Tables 1 and 2.

Unfixed frozen sections of the biopsies were immunohistochemically stained for the presence of dystrophin, vimentin, desmin, laminin and merosin according to Leyten et al. [11]. For laminin and merosin detection, polyclonal rabbit antilaminin (Sanbio, dilution 1:50) and monoclonal anti-human merosin (Gibco, dilution 1:100) were used, respectively.

The polyclonal rabbit antibody was raised against amnion BM and showed a distinct band at 200-220 kDa in immunoblots (probably B1 or B2 side chains). The monoclonal anti-human merosin antibodies reacted with an 80 kDa fragment of the M-chain of human merosin.

To avoid misinterpretations, immunolabelling of control tissue sections from 13 other patients -of the same age and stored for the same length of time- was also performed simultaneously.

Results

Intensity and distribution of polyclonal laminin staining of the intra- and extrafusal muscle fibres (including the spindle capsules), capillaries and small blood vessels, nerves and perineuria (if present) were slightly variable in the CMD and control patients. No significant differences were present.

Merosin expression was limited to muscle fibres (the sarcolemma) and nerves (the Schwann cells, if present). The staining was regular and intense in 6 out of the 8 pure-CMD patients; in the 2 other pure-CMD patients (patients 7 and 8, the patients with white matter hypodensity) merosin staining was markedly decreased (Table 2, Fig. 1A). Merosin staining was normal in the 3 F-CMD patients (Fig. 1B) and in the 2 WWS patients (Table 2).

Dystrophin, vimentin and desmin staining were basically normal in all the patients; vimentin positive fibres were only found occasionally; these represent basophilic fibres in HE staining.

The basement membranes of the muscle fibres in the two patients with decreased merosin were normal (Fig. 1C) and could not be distinguished in any way from controls by electron microscopy.

Discussion

Our merosin deficient patients were classified clinically (by CT/MRI) as "pure" CMD with diffuse white matter abnormalities. A similar correlation was found by Mercuri et al. in 7 patients [15] and by Voit et al. [22] in 1 patient.

Our data on polyclonal laminin were slightly variable in the patients and controls. This could be ascribed to the storage time of the muscle sections, because the longer the sections were stored, the poorer the staining.

The results with respect to normal polyclonal laminin and the low level of monoclonal merosin staining suggested abnormal merosin, but normal B1 and/or B2 chains. The latter is in agreement with (i) the location of B1 and B2 coding genes on chromosomes other than chromosome 6 (viz. 7 and 1), (ii) the normal presence of B1 and B2 laminin subunits in merosin-negative patients as was shown by monoclonal antibodies [21], (iii) the probable B2 specificity of polyclonal laminin antibodies [7], (iv) the self-assembly of laminin by the short arm (B1 and B2) globular domains as demonstrated in vitro [24] and (v) our normal ultrastructural findings in the basement membrane.

Merosin has recently [1] been found to be missing in the *dy/dy* mouse (a strain that suffers from progressive fatal muscular dystrophy). Inheritance in these animals follows an autosomal recessive pattern and both the disease and the merosin locus were shown to be localized within the same region on chromosome 10 [18]. These animals showed an ultrastructurally abnormal basement membrane [1]. The human merosin-negative patients showed an over-expression of laminin A [21]. A possible lack of this compensation mechanism in the animal model might contribute to the abnormal appearance of the basement membrane in the *dy/dy* mouse.

Merosin gene locus and merosin deficient CMD are closely linked [8], which strongly suggests that merosin deficiency is the primary cause of this type of dystrophy.

In this study, normal merosin expression was found in the 3 F-CMD patients. These merosin findings contrast with the findings in 17 Japanese F-CMD patients [7]. On average, there was a 74% reduction in these patients. As the gene for Japanese F-CMD is localized on chromosome 9 [19] while the merosin gene is localized on chromosome 6, these low levels of merosin are probably secondary to another as yet unknown defect. Thus, our results show that phenotypic heterogeneity is present in F-CMD with regard to merosin expression. A similar condition was found in SCARMD for 50 kDa DAG or adhalin expression; adhalin positive and adhalin negative patients were found in a Brazilian study [25]. Furthermore, adhalin deficiency in SCARMD was shown to be linked to chromosome 13q12 [2], while in another family linkage to chromosome 17q12-21.33 -the adhalin locus- was found [17]. The differences between Japanese and non-Japanese F-CMD with regard to merosin expression may be based on genotypic heterogeneity, as is also the case in SCARMD.

The normal merosin results in our non-Japanese F-CMD are in agreement with a normally functioning merosin gene. Our findings indicate that different primary defects may be involved in F-CMD. However, according to Dobyns et al. [4], another explanation for the merosin discrepancy might be that most of the non-Japanese F-CMD patients had to be diagnosed as F-MEB-D or WWS because of their more severe typical anomalies. Gene localization studies in non-Japanese F-CMD families are needed to clarify this.

The merosin status in 2 WWS patients was normal, which agrees with the normal merosin findings in another 5 WWS cases [22].

As genetic identity has been stated for Japanese F-CMD and WWS in the Fukuyama gene region [20], WWS patients can also be expected to show merosin deficiency just as the Japanese F-CMD cases, but this was not the case [22].

Thus, while Toda et al. [20] claimed that F-CMD and WWS were genetically identical, Voit et al. [22] concluded that (based on merosin expression) WWS is a separate disease entity from Japanese F-CMD and also from pure-CMD with merosin deficiency.

Our merosin findings also exclude WWS from being allelic to the latter type of CMD, but they do not exclude genetic identity of WWS and F-CMD.

The question must be left open as to whether WWS and the clinically milder but phenotypically similar Finnish type of MEB-D are allelic disorders. The merosin status of the Finnish type patients has not yet been reported.

References

1. Arahata K, Hayashi YK, Koga R, Goto K, Lee JH, Miyagoe Y, Ishii H, Tsukahara T, Takeda S, Woo M, Nonaka I, Matsuzaki T, Sugita H (1993) Laminin in animal models for muscular dystrophy. Defect of laminin M in skeletal and cardiac muscles and peripheral nerve of the homozygous dystrophic dy/dy mice. Proc Japan Acad 69 (Series B): 259-264.
2. Azibi K, Bachner L, Beckmann JS, Matsumura K, Hamouda E, Chaouch M, Chaouch A, Ait-Ouarab R, Vignal A, Weissenbach J, Vinet M, Leturcq F, Collin H. Tomé FMS, Reghis A, Fardeau M, Campbell KP, Kaplan J (1993) Severe childhood autosomal recessive muscular dystrophy with the deficiency of the 50 kDa dystrophin-associated glycoprotein maps to chromosome 13ql2. Hum Mol Genet 2: 1423-1428.
3. Burgeson RE, Chiquet M, Deutzmann R, Ekblom P, Engel J, Kleinmann H, et al. (1994) A new nomenclature for the laminins. Matrix Biol 14: 209-211.
4. Dobyns WB, Truwit CL (1995) Lissencephaly and other malformations of cortical development: 1995 update. Neuropediatrics 26: 132-147.
5. Dubowitz V (1994) Workshop report: 22nd ENMC sponsored workshop on congenital muscular dystrophy held in Baarn, The Netherlands, 14-16 May 1993. Neuromusc Disord 4: 75-81.
6. Dubowitz V, Fardeau M (1995) Workshop report: proceedings of the 27th ENMC sponsored workshop on congenital muscular dystrophy. 22-24 April 1994, The Netherlands. Neuromusc Disord 5: 253-258.
7. Hayashi YK, Engvall E, Arikawa-Hirasawa E, Goto K, Koga R, Nonaka I, Sugita H, Arahata K (1993) Abnormal localization of laminin subunits in muscular dystrophies. J Neurol Sci 119: 53-64.
8. Hillaire D, Leclerc A, Fauré S, Topaloglu H, Chiannilkulchaï N, Guicheney P, Grinas L, Legos P, Philpot J, Evangelista T. Routon M-C, Mayer M, Pellissier J-F, Estournet B, Barois A, Hentati F, Feingold N, Beckmann JS, Dubowitz V, Tomé FMS, Fardeau M (1994) Localization of merosin-negative congenital muscular dystrophy to chromosome 6q2 by homozygosity mapping. Human Mol Genet 3: 1657-1661.
9. Ibraghimov-Beskrovnaya O, Ervasti JM, Leveille CJ, Slaughter CA, Sernett SW, Campbell KP (1992) Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. Nature 355: 696-702.

10. Ibraghimov-Beskrovnaya O, Milatovich A, Ozcelik T, Yang B, Francke U, Campbell KP (1992) Dystroglycan: tissue distribution, human muscle cDNA, genomic structure and chromosome mapping. Am J Hum Genet 51(Suppl): A130.
11. Leyten QH, Ter Laak HJ, Gabreëls FJM, Renier WO, Renkawek K, Sengers RCA (1993) Congenital muscular dystrophy. A study on the variability of morphological changes and dystrophin distribution in muscle biopsies. Acta Neuropathol 86: 386-392.
12. Matsumura K, Campbell KP (1994) Dystrophin-glycoprotein complex: its role in the molecular pathogenesis of muscular dystrophies. Muscle Nerve 17: 2-15.
13. Matsumura K, Tomé FMS, Collin H, Azibi K, Chaouch M, Kaplan JC, Fardeau M, Campbell KP (1992) Deficiency of the 50K dystrophin-associated glycoprotein in severe childhood autosomal recessive muscular dystrophy. Nature 359: 320-322.
14. Mattei M, Weil D, Pribula-Conway D, Bernard MP, Passage E, Van Cong NG, Timpl R, Chu M (1988) cDNA cloning, expression and mapping of human laminin B2 gene to chromosome 1q31. Hum Genet 79: 235-241.
15. Mercuri E, Muntoni F, Berardinelli A, Pennock J, Sewry C, Philpot J, Dubowitz V (1995) Somatosensory and visual evoked potentials in congenital muscular dystrophy: correlation with MRI changes and muscle merosin status. Neuropediatrics 26: 3-7.
16. Pikkarainen T, Eddy R. Fukushima Y, Byers M, Shows T, Pihlajaniemi T, Saraste M, Tryggvason K (1987) Human laminin B1 chain, a multidomain protein with gene (lamB1) locus in the q22 region of chromosome 7. J Biol Chem 262: 10454-10462.
17. Roberds SL, Leturcq F, Allamand V, Piccolo F. Jeanpierre M, Anderson RD, Lim LE, Lee JC, Tomé FMS, Romero NB, Fardeau M, Beckmann JS, Kaplan J, Campbell KP (1994) Missense mutations in the adhalin gene linked to autosomal recessive muscular dystrophy. Cell 78: 625-633.
18. Sunada Y, Bernier SM, Kozac CA, Yamada Y. Campbell KP (1994) Deficiency of merosin in dystrophic dy mice and genetic linkage of laminin M chain gene to dy locus. J Biol Chem 269: 13729-13732.
19. Toda T. Segawa M, Nomura Y. Nonaka I, Masuda K, Ishihara T, Suzuki M, Tomita I, Origuchi Y, Ohno K, Misugi N, Sasaki Y, Takada K, Kawai M, Otani K, Murakami T, Saito K, Fukuyama Y, Shimizu T, Kanazawa I, Nakamura Y (1993) Localization of a gene for Fukuyama type congenital muscular dystrophy to chromosome 9q31-33. Nature Genet 5: 283-286.

20. Toda T, Yoshioka M, Nakahori Y, Kanazawa I, Nakamura Y. Nakagome Y (1995) Genetic identity of Fukuyama-type congenital muscular dystrophy and Walker-Warburg syndrome. Ann Neurol 37: 99-101.
21. Tomé FMS, Evangelista T, Leclerc A, Sunada Y, Manole E, Estournet B, Barois A, Campbell KP, Fardeau M (1994) Congenital muscular dystrophy with merosin deficiency. CR Acad Sci Paris 317: 351-357.
22. Voit T, Sewry CA, Meyer K, Hermann R, Straub V, Muntoni F, Kahn T, Unsöld R. Helliwell TR, Appleton R, Lenard HG (1995) Preserved merosin M-chain (or laminin-α_2) expression in skeletal muscle distinguishes Walker-Warburg syndrome from Fukuyama muscular dystrophy and merosin-deficient congenital muscular dystrophy. Neuropediatrics 26: 148-155.
23. Vuolteenaho R, Nissinen M, Sainio K, Byers M, Eddy R, Hirvonen H, Shows TB, Sariola H, Engvall E, Tryggvason K (1994) Human laminin M chain (merosin): complete primary structure, chromosomal assignment, and expression of the M and A chain in human fetal tissues. J Cell Biol 124: 381-394.
24. Yurchenco PD, Cheng Y, Colognato H (1992) Laminin forms an independent network in basement membranes. J Cell Biol 117: 1119-1133.
25. Zatz M, Matsumura K, Vainzof M, Passos-Bueno MR, Pavanello RCM, Marie SK, Campbell KP (1994) Assessment of the 50-kDa dystrophin-associated glycoprotein in Brazilian patients with severe childhood autosomal recessive muscular dystrophy. J Neurol Sci 123: 122-128.

Chapter IV

PARTICULAR FORMS OF CONGENITAL MUSCULAR DYSTROPHY

GENERALIZED FORMS OF CONGENITAL MUSCULAR DYSTROPHY

An autosomal dominant type of congenital muscular dystrophy

Q.H. Leyten, F.J.M. Gabreëls, E.M.G. Joosten, W.O. Renier,
H.J. ter Laak, B.G.A. ter Haar, A.M. Stadhouders

Brain & Development 1986;8:533-537

Abstract

A family with an autosomal dominant type of congenital muscular dystrophy (CMD) will be reported. In general, an autosomal recessive mode of inheritance is accepted for CMD. In 1980, Kalyanaraman et al reported another family with an autosomal dominant CMD with possible involvement of the central nervous system (CNS). Our report concerns a father and daughter suffering from CMD without CNS involvement. The histological findings, especially some mitochondrial abnormalities in the muscle biopsy were remarkable.

Congenital muscular dystrophy (CMD) is a rare muscular disorder, characterized by generalized weakness and hypotonia at birth, often with joint contractures and delayed motor development [1-12]. The term CMD was introduced by Howard [2] in 1908. The joint contractures may be limited to talipes or constitute a severe form of arthrogryposis multiplex congenita [1-12]. The onset of this slowly or rapidly progressive muscle weakness occurs in uterine life.

Fukuyama et al [6, 13, 14] differentiate CMD in one type with and one without involvement of the central nervous system (CNS). The type with CNS involvement is also called the Fukuyama type (F-CMD).

In general, an autosomal recessive pattern of inheritance in CMD is suggested [13, 15, 16]. Kalyanaraman et al [17] are the only authors who have reported a family with CMD and possible CNS involvement, suggesting an autosomal dominant pattern of inheritance.

Recently, we had the opportunity to examine a family with CMD without CNS involvement and likely an autosomal dominant mode of inheritance.

Case reports (Fig. 1)

Case 1 (proband)

Patient 1 was a 6-year-old daughter of patient 2, born to unrelated parents, after a normal pregnancy and a delivery by Caesarian section. Birth weight was 3,480 g. Neonatal period was normal. At birth, generalized weakness, hypotonia, flexion contractures of the knees and clubfeet were present.

Further development was characterized by a progressive motor retardation without any mental retardation. At age 3, the girl was able to walk without aid, but fell frequently. At age 5, she was confined to a wheelchair.

At age 6, examination revealed an alert, intelligent thin girl. Extraocular muscles were intact, but there was a mild facial weakness. There was generalized loss of muscle strength in the trunk and limbs. Proximal and distal muscles were affected equally. Tendon reflexes were absent. Bilateral flexion contractures of the elbows, hips, knees and ankles, and a kyphoscoliosis were obvious. Sensory functions were intact.

Biochemical investigations of serum, urine and cerebrospinal fluid (CSF) showed apart from a slightly elevated serum CK (128 U/l, normal <90) no other abnormalities. Lactate and pyruvate levels in serum and CSF were normal as well as blood gas analysis. 24-Hour lactate excretion was in the normal range.

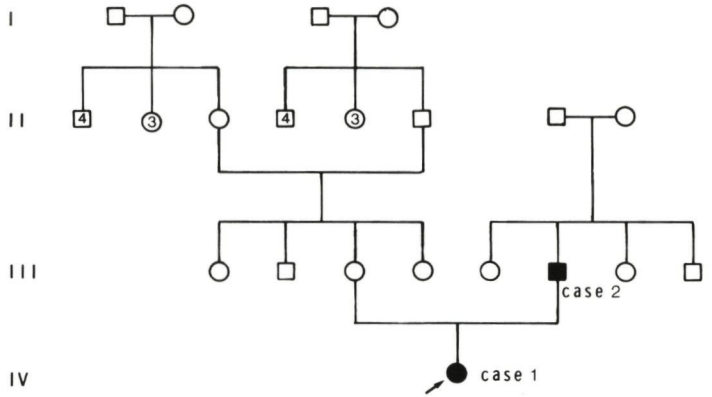

Fig. 1. Pedigree of the family.

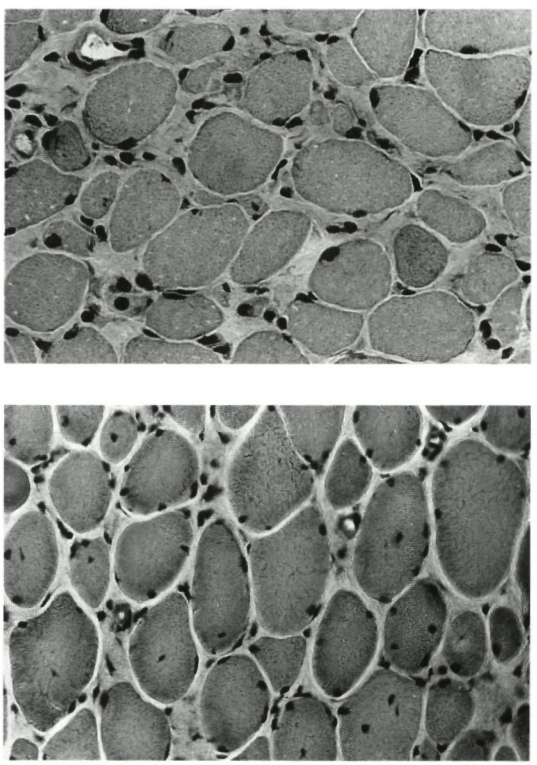

Fig. 2. Transverse sections (HE-stain) of quadriceps muscle biopsy specimens from case 1 (top, x 500) and case 2 (bottom, x 300). In both biopsies there is a considerable variation in muscle fiber diameter. Between the rounded muscle fibers increased quantities of connective tissue can be seen. Note the presence of many muscle fibers with internal nuclei (case 2).

Electromyography (EMG) revealed an increased number of brief polyphasic potentials with small amplitude. Nerve conduction velocities (NCVs) were normal. Electroencephalography (EEG), somatosensory evoked potentials (SSEP), cerebral CT scan, electrocardiography (ECG) and echocardiography revealed no abnormalities.

Case 2

Patient 2 was the father of patient 1, a 36-year-old man, born to unrelated parents after an uncomplicated pregnancy and delivery. Since birth, a generalized hypotonia and gracile musculature was noticed, and wry neck was present. At age 3½ years, he started walking, but generalized hypotonia and muscular atrophy without proximal or distal predominance were present. Gradually, bilateral flexion contractures of the elbows, hips, knees and ankles developed. He was able to walk until the age of 12, when he was confined to a wheelchair.

Examination at age 35 revealed a generalized hypotonia and muscular atrophy. There were severe bilateral flexion contractures of the elbows, hips, knees and ankles with kyphoscoliosis. Sensory functions were normal. Tendon reflexes were absent. There was mild facial weakness. He had a normal intelligence. At age 38, he died because of bilateral bronchopneumonia.

Biochemical investigations of serum (including CK), urine and CSF showed no abnormalities.

EMG revealed an increase in the number of brief polyphasic potentials with a small amplitude. NCVs were normal. EEG, SSEP, cerebral CT scan, ECG and echocardiography revealed no abnormalities.

Muscle biopsies

Case 1 (at age 3 years)

Light microscopy (Fig. 2). A biopsy from quadriceps muscle revealed both increased fat and connective tissue. The ratio of the number of type I and type II fibers (type I/type II) was 2.13. Both types IIA and IIB fibers were observed; 6% of the total number of fibers represented type IIC fibers, most of these were basophilic fibers. Mean diameters of type I and II fibers were about the same (type I: 26 μm ± 9 μm; type II: 25 μm ± 10 μm). Two percent of the fibers, both type I and type II, contained internal nuclei, especially the small type IIC fibers. Phagocytosis was not observed. Isolated basophilic fibers with swollen nuclei were frequently seen. Ragged-red fibers were not observed. The checkerboard pattern was normal and the terminal innervation ratio was also normal.

Table 1. Signs and symptoms in (F-)CMD

	CMD	F-CMD	Cases of	Our cases	
			Kalyanaraman et al [17]	Case 1	Case 2
Early onset hypotonia	+	+	+	+	+
Involvement of facial muscles	+	+	+	+	+
Joint contractures	+	+	+	+	+
Slowly or rapidly progressive course	+	+	+	+	+
Increased CK activity	++	++	++	+	−
Myogenic pattern of EMG	+	+	+	+	+
Dystrophic abnormalities in muscle biopsy	++	++	+	+	+
CNS involvement	−	+	?	−	−

Electron microscopy. Scattered fibers showed a disturbed sarcomere pattern, with loss of myofilaments, enlarged intermyofibrillar spaces and Z-line streaming. The mitochondria in the fibers used to be swollen. Crystalline inclusions were not observed. There were normal quantities of glycogen but the number of neutral lipid droplets was increased. Fibers with folded sarcolemma were suggestive for a repair mechanism after focal necrosis.

Case 2 (at age 35 years)

Light microscopy (Fig. 2). The biopsy (quadriceps muscle) of the father showed a dystrophic pattern. The ratio of the number of type I and type II fibers (type I/type II) was 0.52. Both types IIA and IIB fibers were observed, but there were many intermediate fibers. The mean diameters of type I and type II fibers were about the same (type I: 41 μm ± 12 μm; type II: 43 μm ± 15 μm). About 25 percent of the fibers of both types contained internal nuclei. There was no preference for a particular diameter-range. Ring fibers were present (2%); basophilic fibers were sporadically seen. Fiber fragmentation occurred only in one fiber. Phagocytosis was not observed. Some fibers had increased subsarcolemmal succinic dehydrogenase activity, suggesting the presence of subsarcolemmal mitochondrial aggregates. There were no ragged-red fibers. The checkerboard pattern was not disturbed and the terminal motor nerves in the biopsy were unbranched, which suggests a normal innervating pattern.

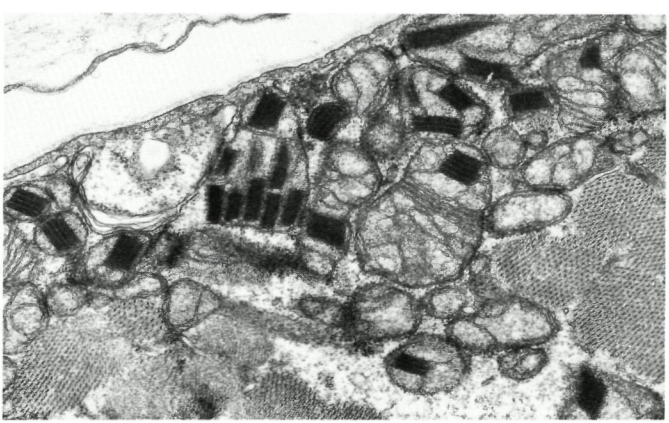

Fig. 3. Electronmicrograph (x 30,000) of quadriceps muscle biopsy specimen of case 2. Severe fibrosis is present around many blood vessels and capillaries. Note the mitochondrial abnormalities such as crystalline inclusions (in type I fibers) and mitochondria with concentric cristae.

Electron microscopy (Fig. 3). Some fibers revealed a focal loss of cross-striation. Many fibers showed mitochondrial abnormalities, such as crystalline inclusions (in type I fibers) and mitochondria with concentric cristae. Fibrosis was severe around blood vessels and the capillaries used to have thickened basal membranes. Many fibers showed small folds intruding formations consisting of basal membranes or connective tissue.

Discussion

Our two cases fulfil the clinical and morphological criteria of CMD, without CNS involvement (see Table 1). Dystrophic changes especially in case 1 are relatively mild. Remarkable is the absence or small increase in CK activity, which, however, has been described more often [8, 9, 12].

Kalyanaraman et al [17] described an autosomal dominant congenital muscular dystrophy (mother and son) with possible CNS involvement (see Table 1). Muscle biopsy showed mitochondrial abnormalities, such as duplication and an increased number of mitochondria of abnormal size and shape with distorted cristae.

In our patients, EM examination of the muscle biopsy of the father showed mitochondria with crystalline inclusions in a large group of fibers and proliferation of concentric cristae. In contrast, EM examination of the muscle biopsy of the daughter revealed no mitochondrial abnormalities. In literature, several types of changes are mentioned in EM examination of muscle biopsies in CMD [18-21]. All these changes, however, are not pathognomonic for CMD, but are interpreted as atypical degenerative characteristics.

Fukuyama et al [13] found mitochondrial anomalies in EM examination of the muscle biopsy in F-CMD, i.e. disappearance of shape and structure.

In 1960, Fukuyama et al [6] proposed an autosomal recessive heredity as the most likely mode of inheritance in CMD. Osawa [22] demonstrated that F-CMD is induced by homozygote conjugation of autosomal recessive genes. In 1980, Kalyanaraman et al [17] described an autosomal dominant type of CMD with possible CNS involvement. CNS involvement was a functional disorder, as in their case the mother had epilepsy with generalized tonic clonic and absence seizures from the age of 14 and her son had also spike and wave complexes in his EEG at the age of 9. Their CT scans were normal. Possibly dystrophy and epilepsy were unrelated.

Considering the mode of inheritance and the absence of CNS involvement in our family, a peculiar type of CMD is probable. Theoretically, the mother of case 1 (see Fig. 1) can be carrier of the autosomal recessive type of CMD. This hypothesis seems very unlikely because of the low frequency of carriers and lack of consanguinity in this family. We believe that our cases represent an autosomal dominant inheritance of CMD but without CNS involvement.

The varying mode of inheritance and the differences in EM findings in CMD may suggest CMD is not one single nosological entity but rather a syndrome.

References

1. Oppenheim H. Über allgemeine und lokalisierte Atonie der Muskulatur (Myotonie) im frühen Kindesalter. *Mschr Psychiat Neurol* 1900;**8**:232.
2. Howard R. A case of congenital defect of the muscular system (Dystrophia muscularis congenita) and its association with congenital talipes equinovarus. *Proc Roy Soc Med* 1908:**1**:157-66.
3. Ullrich O. Kongenitale, atonisch-skelerotische Muskeldystrophie. *Mschr Kinderheilk* 1930;**47**:502-10.
4. Gubbay SS, Walton JN, Pearce GW. Clinical and pathological study of a case of congenital muscular dystrophy. *J Neurol Neurosurg Psychiatry* 1966;**29**:500-8.
5. Vassella F, Mumenthaler M, Rossi E. Congenital muscular dystrophy. In: Walton JN, Canal N, Scarlato G, eds. *Muscle disease.* Proceedings of an international congress, Milan 19-21 May 1969. Excerpta Medica International Congress Series 1970;**199**:620-3.
6. Fukuyama Y, Kawazura M, Haruna H. A peculiar form of congenital progressive muscular dystrophy. Report of fifteen cases. *Pediatr Univ Tokyo (Tokyo)* 1960;**4**:5-8.
7. Otto HF, Lücking T. Kongenitale Muskeldystrophie. Licht- und elektronenmikroskopische Befunde. *Virchows Arch (Pathol Anat)* 1971;**352**:324-39.
8. Donner M, Rapola J, Somer H. Congenital muscular dystrophy: a clinicopathological and follow-up study of 15 patients. *Neuropädiatrie* 1975;**6**:239-58.
9. Rotthauwe HW, Kowalewski S, Mumenthaler M. Kongenitale Muskeldystrophie. *Z Kinderheilk* 1969;**106**:131-62.
10. Ketelsen UP, Freund-Mölbert E, Beckmann R. Klinische und ultrastrukturelle Befunde bei kongenitaler Muskeldystrophie. *Mschr Kinderheilk* 1971; **119**:586-92.
11. Krijgsman JB, Barth PG, Stam FC, Slooff JL, Jaspar HHJ. Congenital muscular dystrophy and cerebral dysgenesis in a Dutch family. *Neuropädiatrie* 1980;**11**:108-20.
12. McMenamin MB, Becker LE, Murphy MB. Congenital muscular dystrophy. A clinicopathologic report of 24 cases. *J Pediatr* 1982;**100**:692-7.
13. Fukuyama Y, Osawa M, Suzuki H. Congenital progressive muscular dystrophy of the Fukuyama type. Clinical, genetic and pathological considerations. *Brain Dev (Tokyo)* 1981;**3**:1-29.

14. Yoshioka M, Okuno T, Ito M, Konishi Y, Itagaki Y, Sakamoto Y. Congenital muscular dystrophy (Fukuyama type). Repeated CT studies in 19 children. *Comput Tomogr* 1981;**5**:81-88.
15. Serratrice G, Cros D, Pellisier J-F, Gastaut J-L, Pouget J. Dystrophie musculaire congenitale. *Rev Neurol (Paris)* 1980:**136**:445-72.
16. McKusick VA. *Mendelian Inheritance in Man.* Johns Hopkins University Press, 1983, pp 846-7.
17. Kalyanaraman K, Kalyanaraman UP, Lee RH. Autosomal dominant congenital muscular dystrophy (CMD) with possible central nervous system (CNS) involvement. *J Neuropathol Exp Neurol* 1980;**39**:366.
18. Misugi N. Light and electron microscopic studies of congenital muscular dystrophy. *Brain Dev (Tokyo)* 1980;**2**:191-9.
19. Ito K. Electron microscopic study of human dystrophic muscle. *Clin Neurol (Tokyo)* 1968;**8**:685-92.
20. Nonaka I, Miyoshino S, Miike T, Ueno T, Usuku G. An electron microscopical study of the muscle in congenital muscular dystrophy. *Kumamoto Med J* 1972;**25**:68-82.
21. Tanimura R. Ultrastructural study of skeletal muscle in floppy infant syndrome – with special emphasis on the cases of central nervous system disorders with marked hypotonia. *Acta Paediatr Jpn (Tokyo)* 1974;**78**:470-80.
22. Osawa M. A genetical and epidemiological study on congenital progressive muscular dystrophy (Fukuyama type). *J Tokyo Women's Med Coll (Tokyo)* 1978;**48**:112-49.

Congenital muscular dystrophy and severe central nervous system atrophy in two siblings

Q.H. Leyten, P.G. Barth, F.J.M. Gabreëls, K. Renkawek, W.O. Renier, A.A.W.M. Gabreëls-Festen, H.J. ter Laak, M.G. Smits

Acta Neuropathologica 1995, in press

Abstract

Severe degenerative features of the nervous system of a hitherto unknown kind, associated with a neuromuscular disorder with histopathological features of congenital muscular dystrophy, are reported in two female siblings. The clinical profile was characterized by generalized hypotonia followed by spastic tetraplegia, contractures, polyneuropathy, lack of cognitive development and progressive microcephaly. There was no involvement of the eyes. Neuropathological examination of the brain of one sibling, who died at the age of 30 months, revealed subtotal loss of neurons in the cerebral and cerebellar cortex and in the ventral pons, and secondary loss of myelin in the cerebral and cerebellar subcortical white matter. Sural nerve biopsy in the other sibling, who had a similar neurological affection, showed a lack of large myelinated fibers.

System atrophy of the central nervous system (CNS) with perinatal onset, and subtotal loss of neurons, predominating in the cerebral and cerebellar cortex and the ventral pons is extremely rare [15]. Congenital muscular dystrophy (CMD) has recently been classified into four categories (CMD I, II, III and IV) [7, 8]. In each category the CNS can be involved. This involvement is mild in a subtype of CMD I, which is associated with white matter hypodensities and deficient merosin status [7, 8, 25, 35]. In gene linkage studies the merosin locus was found on chromosome 6q [8]. CNS involvement is severe in CMD II, which is characterized by moderate neocortical and cerebellar cortical dysplasia as well as white matter abnormality, as in Fukuyama syndrome (F-CMD) [7, 8, 10, 11]. The gene responsible for typical F-CMD was recently mapped to chromosome 9_q31-33 [34]. CNS involvement is also present in CMD III, the Finnish type of muscle-eye-brain disease, which is characterized by hydrocephalus and cobblestone (previously type II) lissencephaly, as in CMD IV, Walker-Warburg syndrome, with similar but more severe brain abnormalities [4, 7, 8, 19-21, 27, 29, 36].

We describe two siblings who had severe cerebral system atrophy together with CMD and a lack of large myelinated fibers in peripheral nerve.

Case Reports

Case 1

A girl, the fourth child of healthy Turkish parents, was born after an uneventful pregnancy and birth. Consanguinity could not be proved. The older two children of the family – both girls – appeared to be healthy. The family history did not mention neuromuscular diseases or mental retardation. Birth weight was 3330 g. Head circumference at birth was 35 cm [50th percentile (P50)], but at the age of 3 months 38 cm (<P2.5), indicating a secondary microcephaly. The postpartum period was not complicated by asphyxia. From birth, generalized muscle weakness including facial muscles, head lag and atrophy of hypothenar were present. Deep tendon reflexes and the sucking reflex were absent. Further development was complicated by severe psychomotor retardation. The infant reacted to auditory and tactile stimuli, but not to visual ones. Ophthalmological examination revealed uncontrolled eye movements, but no structural eye abnormalities. In the first few months, hypertonia was noted, especially involving the legs. The infant did not reach any motor milestone. At the age of 30 months, she died from respiratory insufficiency. Autopsy was performed immediately after death.

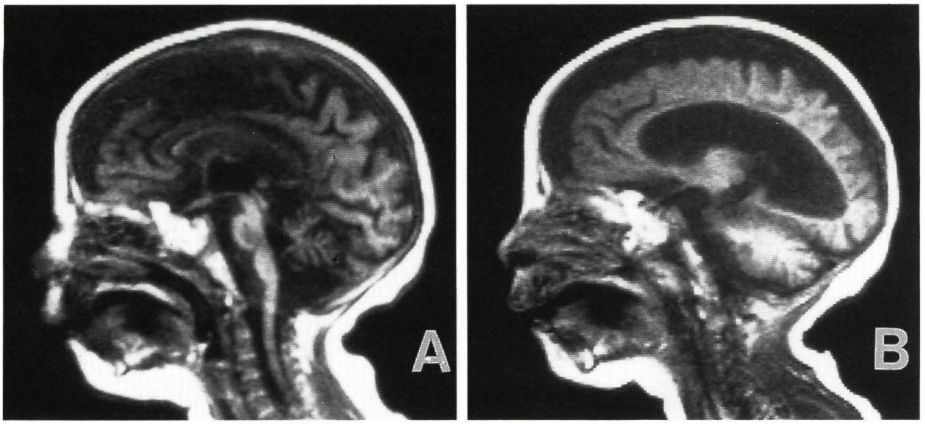

Fig. 1. Magnetic resonance imaging (case 1) at the age of 7 months revealing severe cerebral and cerebellar cortical atrophy as well as hypomyelination (A) and ventricular widening (B).

Biochemical investigations of serum and urine gave normal values except for increased creatine kinase (CK: 527 U/l, normal <90 U/l). In the CSF, no abnormalities could be found. Appropriate studies ruled out metabolic, endocrinological, immunological, and chronic infectious diseases, vitamin deficiencies, and disorders caused by toxic agents. Microbiological examination excluded infections such as toxoplasma, cytomegalovirus, herpes or rubella virus. Chromosome analysis revealed a normal 46 XX karyotype. EEG showed a diffuse slowing of the background pattern with spike and wave paroxysms over the left hemisphere. EMG revealed myopathic characteristics and decreased motor nerve conduction velocities (MNCVs) of the median nerve (25 m/s at the age of 7 months; normal 35-58 m/s). Brainstem auditory evoked potential (BAEP), visual evoked potential (VEP) and somatosensory evoked potential (SSEP) recordings at the age of 4 months were isoelectric. Cerebral computed tomography (CT) and magnetic resonance imaging (MRI) at the age of 7 months revealed ventricular widening and severe cerebral and cerebellar cortical atrophy as well as hypomyelination (Fig. 1). EMG performed in both parents did not show any abnormality.

Muscle biopsy

Eight muscle samples were available for morphological study one sample was taken at 4 months from the quadriceps muscle and the others were taken at autopsy from the diaphragm, the intercostalis externus and rectus abdominis muscles, and from the left and right psoas and iliopsoas muscles Routine histochemical and histopathological stains were done on rapidly frozen sections from the biopsy specimen, including H&E, Gomori trichrome, Ca-ATPase at pH 10 3 and after preincubation at pH 4 6 and pH 4 3, NADH-tetrazolium oxidoreductase, acid phosphatase, periodic acid Schiff (PAS), and Sudan black B Immunohistochemistry included stains for dystrophin, vimentin, desmin, and merosin Muscle samples taken at autopsy were prepared for paraffin sections

The quadriceps biopsy showed a normal percentage of type I fibers, many fibers of both types, increase of muscle fiber diameter variation, and a moderate endomysial fibrosis (Fig 2A) Scattered small basophilic fibers with swollen nuclei showed an increased acid phosphatase activity There were some slightly hypertrophic type I fibers, but there was no bimodal fiber diameter distribution, nor type grouping, nor small or large group atrophy Immunohistochemistry with dystrophin, vimentin, and desmin antibodies revealed a normal pattern of expression However, antibodies against merosin did show a variable immunoreactivity The other seven muscle specimens taken at autopsy 2 years later showed essentially the same muscle fiber diameter variation and fibrosis, but now a moderate to heavy fat cell infiltration was present

Pathological examination

General autopsy revealed marked muscle atrophy and contractures of joints Organ malformations were not present Slight splenomegaly and microscopical infectious changes in the spleen and lungs were found

Neuropathological examination

Formalin-fixed, paraffin-embedded samples from frontal, central, parietal, temporal and occipital cortex, hippocampus, cerebellum, brain stem and spinal cord were cut at 6μm sections and stained with hematoxylin-eosin, Luxol fast blue, trichrome, and Bodian methods Immunohistochemical reactions for neuron-specific enolase (NSE), neurofilaments, glial fibrillar acidic protein (GFAP), vimentin, carbonic anhydrase and myelin basic protein were performed according to the avidin-biotin complex (ABC) method [13]

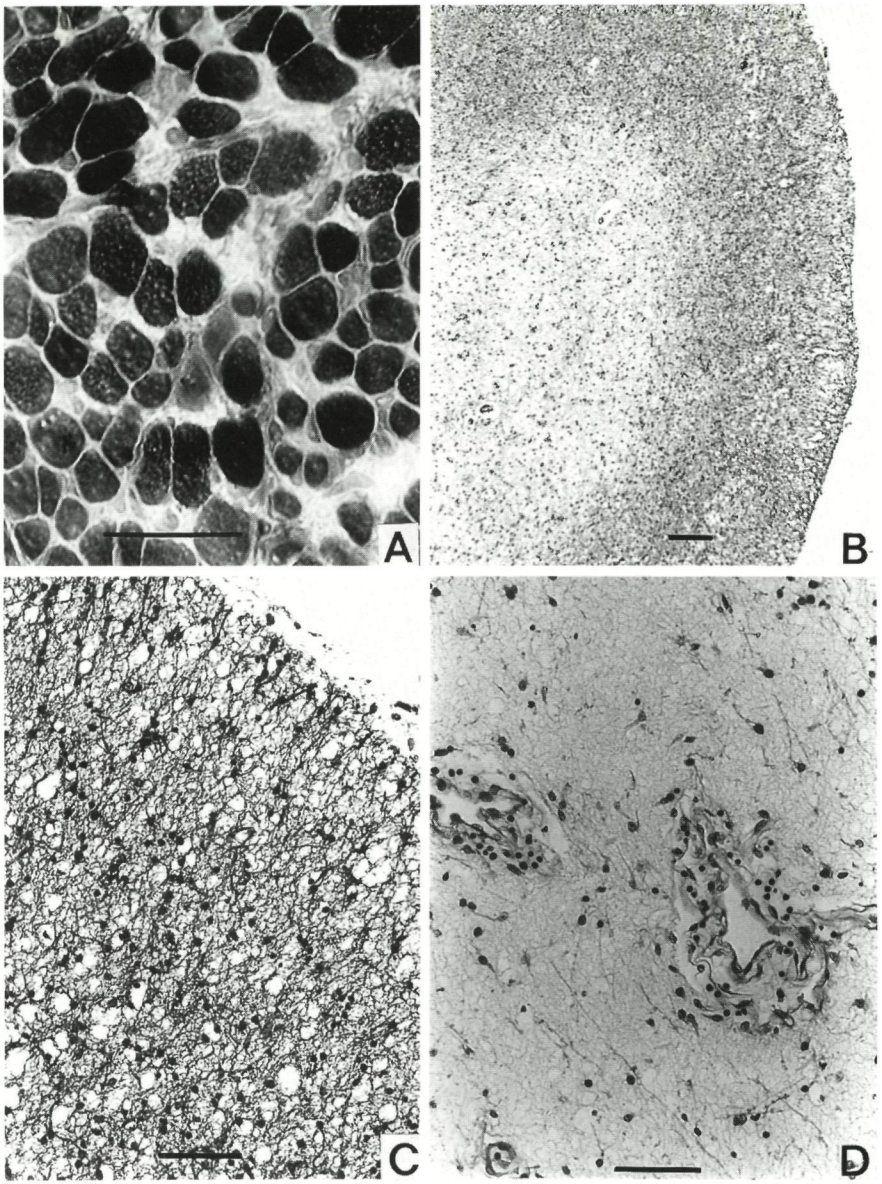

Fig. 2. **A** Quadriceps muscle (case 1) at the age of 4 months demonstrating different size of the muscle fibers and endomysial fibrosis. Gomori trichrome. **B** Frontal lobe showing complete destruction of the neocortex and lack of subcortical myelin. Luxol fast blue-cresyl violet. **C** Cerebral cortex replaced by astrocytic cells. GFAP immunoreaction. **D** Temporal cortex. Scanty myelinated fibers in the white matter. Proliferating mesenchymal tissue of the vessels surrounded by glial cells. Staining for myelin basic protein.
Bars **A, C, D** = 50 μm; **B** = 100 μm.

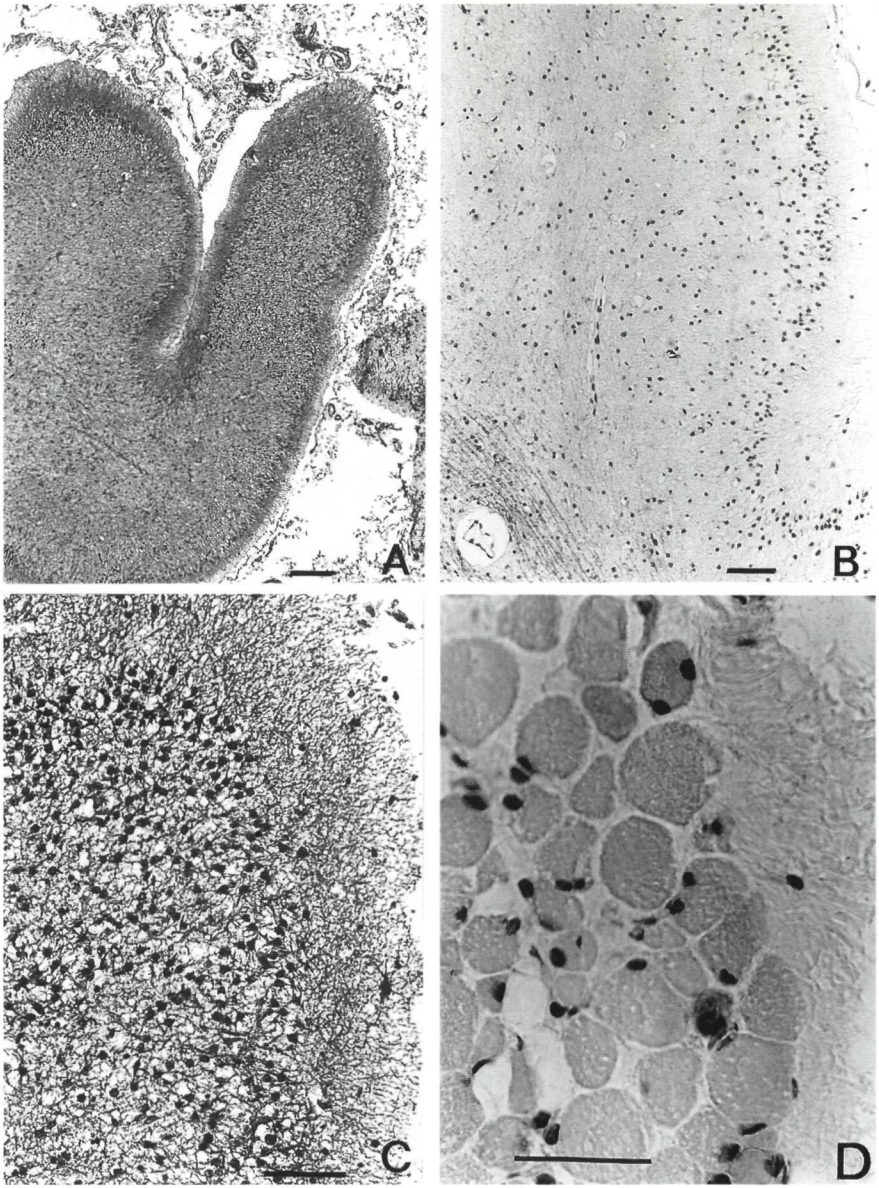

Fig. 3. **A** Cerebellar foliae surrounded by thickened meninges. Loss of neurons and proliferation of glial fibrils. Phosphotungstic acid-hematoxylin staining. **B** Cerebellum. Narrow band of granular neurons, lack of Purkinje cells, myelin and lack of neurofilaments. Staining for the 60-kDa neurofilament. **C** Replacement of cerebellar neuronal layers by glial cells. GFAP staining. **D** Quadriceps muscle (case 2) at the age of 16 months showing variable muscle fiber sizes, endomysial fibrosis and a basophilic fiber. H&E stain.
Bars **A** = 100 μm; **B-D** = 50 μm.

Gross examination revealed a brain weight of 225 g (normal for age 900 g). The leptomeninges were slightly opaque, particularly in the basal cisterns, over the cerebellum and along the fissures. Cerebral and cerebellar surfaces showed pronounced atrophy. Primary and secondary gyri were poorly distinguishable. The olfactory bulbs, tracts, optic nerves, mammillary bodies, cerebral peduncles and brain stem were reduced in size. Cranial nerves were macroscopically unremarkable. Cerebellar hemispheres and vermis were small but well shaped. On the coronal sections, poorly delineated gray and white matter, underdeveloped hippocampi, basal ganglia and thalami, thin callosal body, and dilated ventricles were seen.

Cerebral cortex (temporal neocortex, hippocampal gyrus). Subtotal depletion of neurons was seen, with NSE only staining the fifth layer (Fig. 2B). Layers II, III and IV had virtually disappeared. Layer VI was partially preserved. Replacement gliosis was found (Fig. 2C). The hippocampal cortex was only represented by the hippocampal gyrus, for which a number of neurons was identifiable. Other neocortical areas showed similar abnormalities, characterized by extreme depletion of neurons. In some areas, some preservation of NSE-positive cells in the area corresponding to the second layer was visible. The layer corresponding to the fifth layer and the one or two layers above it were affected by a sponge-like change, but this was not evident in all the sections. The subcortical white matter showed a diffuse lack of myelinated fibers (Fig. 2D). No demyelinating lesions were found.

Cerebellum. There was almost total depletion of the interior granular layer and total disappearance of the Purkinje cell layer (Fig. 3A). A few remaining granule cells and cells of glial lineage could be easily differentiated by NSE. The molecular layer was curiously divided in a lower half which was GFAP positive and NSE negative, and an upper half which was GFAP negative and NSE positive. Therefore, an appreciable number of fibers in the molecular layer were lost and replaced by Bergmann glia. The intrafolial white matter was represented by large GFAP- and S-100 protein-positive fibrous astrocytes, and myelinated fibers were few (Figs. 3B, C).

Basal ganglia. Basal ganglia showed undifferentiated structures and few neurons showed correctly preserved features of large neurons and positive reactions for NSE. The nucleus caudatus was poorly developed. There were abundant vessels in the basal ganglia and in the meninges.

Pons Myelin was present in the medial longitudinal fasciculus, lateral and medial lemnisci, and conjunctival brachia, but almost complete absent in the ventral pons The ventral pons also displayed severe loss of neurons

Medulla oblongata The inferior olivary nucleus was depleted of neurons No reactive gliosis was encountered Well-developed myelin was present in the restiform bodies, medial lemnisci, and spinal trigeminal tracts, but was absent in and around the inferior olivary nuclei and the pyramidal tracts

Cervical spinal cord The lateral corticospinal tracts could not be traced and were either unmyelinated or completely absent The dorsal spinocerebellar tracts were underdeveloped There was no reactive gliosis Only the medial group of motor neurons could be identified No sections of spinal ganglia were available

In the brain stem and cervical spinal cord there were no other pathological changes

Case 2

The older sister of case 1, the third child of the parents, was born after an uneventful pregnancy of 41 5 weeks in breech presentation Birth weight was 3830 g (P60) Head circumference was 35 cm at birth (P50), but at the age of 3 months was 38 cm (<P2 5) The postpartum period was not complicated by asphyxia The same clinical signs and symptoms as in case 1 occurred (head lag, atrophy of hypothenar, absent deep tendon reflexes, absent sucking reflex) The girl was also profoundly retarded She reacted to auditory and tactile stimuli, but not to visual ones Ophthalmological examination confirmed lack of control of eye movements and lack of structural eye abnormalities

In the first few months, progressive pyramidal signs developed leading to contractures, especially in knee and ankle joints The muscular system appeared to be hypoplastic, especially in the distal muscles At the age of 21 months, she suddenly died, probably of a pulmonary infection Autopsy was not allowed

Findings of biochemical investigations of serum and urine were similar to those in case 1, and all normal, except CK (904 U/l) Chromosome analysis revealed a normal 46 XX karyotype EEG showed a diffuse slowing of the background pattern EMG revealed an increased number of brief polyphasic potentials with small amplitude MNCVs of the median and peroneal nerve were markedly decreased (15 m/s at the age of 4 months, normal 37-48 m/s, and 18 m/s at the age of 17 months, normal 39-54 m/s) BAEP, VEP and SSEP recordings at the age of 17 months were isoelectric Cranial CT showed enlarged ventricles and cerebral and cortical cerebellar atrophy

Muscle biopsy

Biopsy specimens from the quadriceps (two samples) and soleus muscle were taken at 6 and 16 months after birth. The picture (Fig. 3D) was clearly dystrophic with many small rounded fibers of both types, moderate endomysial fibrosis, some scattered basophilic fibers, and fibers with internal nuclei. The percentage of type I fibers was 80% (both quadriceps muscles) and 40%, respectively. Type grouping and small or large group atrophy were absent. The muscle fiber diameter distribution was unimodal and hypertrophy was only observed in the quadriceps muscles. In the latter two biopsies samples (taken at 16 months) a heavy fat cell infiltration was found. Except for basophilic fibers, no other fibers were acid phosphatase positive; PAS stain was normal. Electron microscopy of the soleus muscle did not show abnormal lipofuscin or other indications for storage disease. Cross-striations were often disturbed. Dilated sarcoplasmic reticulum, proliferating T tubules, autophagic vacuoles, and mitochondria with poor cristae were found. Fibers were sometimes surrounded by collagen. Basal lamina did not generally show redundant loops, the latter excluding fast volume decreases as seen in acute infantile spinal muscular atrophy. Immunohistochemistry with dystrophin, vimentin, desmin, and merosin antibodies revealed a normal pattern of expression in the soleus muscle.

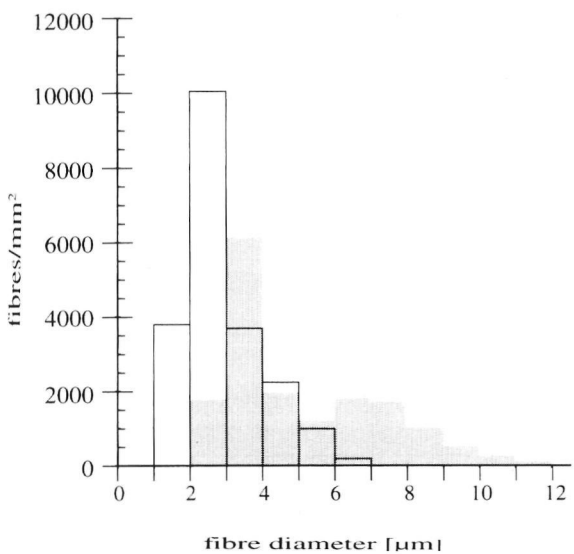

Fig. 4. Sural nerve (case 2). Distribution of diameters of myelinated fibers expressed in density per mm^2 (*white area*) compared with age-matched control (*hatched area*).

Sural nerve biopsy

Qualitative light and electron microscopical examination did not reveal pathology. However, quantitative examination of the nerve biopsy revealed some differences from the normal. There was a slightly increased density of myelinated fibers (20,890 fibers/mm^2, mean of age-matched controls 15,660 fibers/mm^2, range 11,860-19,520 fibers/mm^2). The total number of myelinated fibers was normal (8560 fibers, mean of age-matched controls 8050 fibers, range 4750-10,540 fibers). Large-diameter fibers were almost lacking (Fig. 4). The proportion of fibers with a diameter of greater than 6 μm was less than 0.5%. In five control nerves of children between 1 and 2 years of age, it was 27% (range 22-30%). The myelinated fiber histogram showed a shift towards smaller diameter fibers and a peak of the histogram between 2 and 3 μm. The thickness of the myelin sheath compared to axon diameter was normal [30], which rules out axon atrophy as possible explanation for the lack of large fibers. We found no signs indicative of a degenerating process; thus, we consider a maturation defect the most likely explanation of the pathological features.

Discussion

Both siblings presented an identical clinical, neurophysiological and neuroradiological profile. At birth, they had a generalized hypotonia, followed after several weeks by spastic quadriplegia and at later stages joint contractures. Further investigations revealed a reduction in nerve conduction velocities, a myopathic EMG pattern, and mildly increased CK. Both children developed a secondary microcephaly with large cerebral ventricles. They were severely mentally retarded.

Histomorphological and histochemical examination of muscle biopsies of both patients revealed characteristics of CMD [5, 6, 10, 11, 18, 19, 22, 24, 31]. Immunohistochemistry with dystrophin, vimentin, desmin, and merosin antibodies showed a normal pattern of expression, except for a variable immunoreactivity with merosin antibodies in case 1.

A quantitative neuropathological examination of sural nerve showed a normal total number of myelinated fibers but a lack of large-diameter fibers. A pathogenic mechanism for this lack of large-diameter fibers could not be established, but may be part of a global maturation defect of the peripheral nervous system.

Neuropathological examination of the CNS in case 1 revealed a severe depletion of cerebral and cerebellar neurons and fibrous gliosis, pointing to an early

degenerative process The ventral pons, the inferior olivary nucleus, and the lateral group of motor neurons in the cervical spinal cord were also depleted of neurons The myelin deficiency was restricted to the area around the inferior olivary nuclei and to the pyramidal tracts in the medulla oblongata and the lateral corticospinal tracts in the cervical spinal cord This was due to loss of corresponding cerebral and cerebellar neurons

The present disorder, in as much as the brain is concerned, has characteristics of a primary and progressive neurodegenerative disorder of gray matter with very early onset In this regard there are parallels with earlier publications, such as the multisystem degeneration of prenatal onset described by Kaarsoo Herrick et al [15], and the pontocerebellar hypoplasias by Barth [2] However, in the cases of these authors no muscular dystrophy, or a lack of large-diameter fibers in sural nerve morphology was mentioned In the literature on CMD and other muscular dystrophies associated with pathological findings in the CNS (Table 1), we could not find any similar cases Especially the severity of the brain pathology and the developmental defect of the peripheral nerve are striking

Table 1. Muscular dystrophy associated to brain pathology in men

- Myotonic dystrophy [12]
- Duchenne's / Becker's muscular dystrophy [6, 28]
- Congenital muscular dystrophy with severe impairment of intellectual development (Fukuyama type of CMD, Walker-Warburg syndrome and Finnish muscle-eye-brain disease) [4, 7, 8, 10, 11, 19-21, 27, 29, 36], Fowler's syndrome [3]
- Muscular dystrophy in Marinesco-Sjogren syndrome [17]
- Lethal congenital muscular dystrophy with cataracts and a minor brain anomaly [37]
- Congenital adrenal hypoplasia, muscular dystrophy, and glycerol kinase deficiency [9]

Comparing our patients with F-CMD (CMD II), the following differences are noted

1 The neuropathological findings in our patient are more severe than those described until now in F-CMD [10, 11], especially the complete loss of normal cytoarchitecture of the cortex of cerebrum and cerebellum, and the myelin deficiency In F-CMD, a mild gliosis or edema of the cerebral white matter or

myelin pallor in the centrum semiovale has been reported [16, 33], but in these series most F-CMD cases showed no remarkable abnormalities in the cerebral white matter.

2. Microcephaly in our patients (<P2.5) is more severe. In the review article of Fukuyama et al. [11], microcephaly in 13 females amounts to the 'min SD' level.

3. At birth our patients presented generalized weakness and hypotonia, but later on developed a progressive hypertonia in contrast to the generalized hypotonia in F-CMD patients [10, 11].

4. Neuroradiological findings on CT/MRI in our patients (i.e., enlarged ventricles, cerebral atrophy and severe generalized decreased white matter lucency in the cerebrum) are different from those described in F-CMD patients by Yoshioka et al. [38-40], who described delayed myelination especially in the frontal lobes, which are the last areas to be myelinated [1]. Moreover, they mentioned that the low-density areas in the cerebral white matter were most apparent around the age of 1 year and that they decreased or disappeared by 2-3 years of age [38, 39]. These observations suggest that the low-density areas found in F-CMD on CT/MRI are due to a delay in myelination rather than to a demyelination or a dysmyelination process [40].

5. Neurophysiological and neuromorphological studies of the peripheral nervous system of our patients manifest a lack of large-diameter fibers, a finding not described in F-CMD [10, 11]. Neuromorphological studies of spinal anterior roots and medullated nerve fibers in F-CMD did not show any deviation from those in the controls with respect to the total number as well as the distribution of fiber diameter, and no pathological findings such as segmental demyelination, myelin bulb formation or axonal degeneration could be found in nerve fibers by the teasing method [11, 14, 32]. However, one should be cautious about drawing conclusions from these differences because long-term follow-up of several F-CMD cases revealed marked individual and intrafamilial variability [26]. If our cases are considered as F-CMD, then they illustrate a most severe type. For further differentiation within the F-CMD variability, staining for dystrophin-associated proteins [23] and molecular genetic studies ('genetic mapping'), especially on chromosome 9 [34], is necessary.

In conclusion, the brain and sural nerve pathology of our patients essentially differ from those described previously in conjunction with CMD. We consider the pathological changes to be a very early multisystem degeneration of the CNS and a developmental defect of the peripheral nervous system. An autosomal recessive mode of inheritance is likely.

References

1. Barkovich AJ, Kjos BO, Jackson DE Jr, Norman D (1988) Normal maturation of the neonatal and infant brain: MR imaging at 1.5 T. Radiology 166: 173-180
2. Barth PG (1993) Pontocerebellar hypoplasias: an overview of a group of inherited neurodegenerative disorders with fetal onset. Brain Dev 15:411-422
3. Dobyns WB (1993) Classification of the cerebro-oculo-muscular syndrome(s). Commentary to Kimura's paper (pp 182-191) (comment). Brain Dev 15:242-244
4. Dobyns WB, Pagon RA, Armstrong D, Curry CJR, Greenberg F, Grix A, Holmes LB, Laxova R, Michels VV, Robinow M, Zimmerman RL (1989) Diagnostic criteria for Walker-Warburg syndrome. Am J Med Genet 32: 195-210
5. Donner M, Rapola J, Somer H (1975) Congenital muscular dystrophy: a clinico-pathological and follow-up study of 15 patients. Neuropädiatrie 6: 239-258
6. Dubowitz V (1978) Muscle disorders in childhood. Saunders, London
7. Dubowitz V (1994) Workshop report: 22nd ENMC sponsored workshop on congenital muscular dystrophy held in Baarn, The Netherlands, 14-16 May 1993. Neuromusc Disord 4:75-81
8. Dubowitz V, Fardeau M (1995) Workshop report: proceedings of the 27th ENMC sponsored workshop on congenital muscular dystrophy. 22-24 April 1994, The Netherlands. Neuromusc Disord 5:253-258
9. Francke U, Harper JF, Darras BT, Cowan JM, McCabe ERB, Kohlschütter A, Seltzer WK, Saito F, Goto J, Harpey J-P, Wise JE (1987) Congenital adrenal hypoplasia, myopathy, and glycerol kinase deficiency: molecular genetic evidence for deletions. Am J Hum Genet 40:212-227
10. Fukuyama Y, Kawazura M, Haruna H (1960) A peculiar form of congenital progressive muscular dystrophy: report of fifteen cases. Pediatr Univ Tokyo (Tokyo) 4:5-8
11. Fukuyama Y, Osawa M, Suzuki H (1981) Congenital progressive muscular dystrophy of the Fukuyama type: clinical, genetic and pathological considerations. Brain Dev 3:1-29
12. Harper PS (1989) Myotonic dystrophy, 2nd ed. Saunders, Philadelphia

13. Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577-580
14. Iida M, Iwata M, Bando M, Kurisaki H (1980) A consideration on the pathogenesis of brain pathology in Fukuyama type congenital muscular dystrophy. In: Sobue I (ed) The 1979 annual report from the research group on the clinico-pathology and epidemiology of progressive muscular dystrophy. Ministry of Health and Welfare of Japan, Tokyo, pp 385-387
15. Kaarsoo Herrick M, Strefling AM, Urich H (1983) Intrauterine multisystem atrophy in siblings: a new genetic syndrome? Acta Neuropathol (Berl) 61: 65-70
16. Kamoshita S, Konishi Y, Segawa M, Fukuyama Y (1976) Congenital muscular dystrophy as a disease of the central nervous system. Arch Neurol 33: 513-516
17. Komiyama A, Nonaka I, Hirayama K (1989) Muscle pathology in Marinesco-Sjögren syndrome. J Neurol Sci 89:103-113
18. Leyten QH, Gabreëls FJM, Joosten EMG, Renier WO, Ter Laak HJ, Ter Haar BGA, Stadhouders AM (1986) An autosomal dominant type of congenital muscular dystrophy. Brain Dev 8:533-537
19. Leyten QH, Gabreëls FJM, Renier WO, Ter Laak HJ, Sengers RCA, Mullaart RA (1989) Congenital muscular dystrophy. J Pediatr 115:214-221
20. Leyten QH, Renkawek K, Renier WO, Gabreëls FJM, Mooy CM, Ter Laak HJ, Mullaart RA (1991) Neuropathological findings in muscle-eye-brain disease (MEB-D): neuropathological delineation of MEB-D from congenital muscular dystrophy of the Fukuyama type. Acta Neuropathol 83:55-60
21. Leyten QH, Gabreëls FJM, Renier WO, Renkawek K, Ter Laak HJ, Mullaart RA (1992) Congenital muscular dystrophy with eye and brain malformations in six Dutch patients. Neuropediatrics 23:316-320
22. Leyten QH, Ter Laak HJ, Gabreëls FJM, Renier WO, Renkawek K, Sengers RCA (1993) Congenital muscular dystrophy: a study on the variability of morphological changes and dystrophin distribution in muscle biopsies. Acta Neuropathol 86:386-392
23. Matsumura K, Nonaka I, Campbell KP (1993) Abnormal expression of dystrophin-associated proteins in Fukuyama-type congenital muscular dystrophy. Lancet 341:521-522
24. McMenamin JB, Becker LE, Murphy EG (1982) Congenital muscular dystrophy: a clinicopathologic report of 24 cases. J Pediatr 100:692-697

25. Mercuri E, Muntoni F, Berardinelli A, Pennock J, Sewry C, Philpot J, Dubowitz V (1995) Somatosensory and visual evoked potentials in congenital muscular dystrophy: correlation with MRI changes and muscle merosin status. Neuropediatrics 26:3-7
26. Osawa M (1994) Fukuyama type congenital muscular dystrophy (FCMD): clinical, pathological and genetic approaches (abstract). Muscle Nerve (Suppl 1):S53
27. Raitta C, Lamminen M, Santavuori P, Leisti J (1978) Ophthalmological findings in a new syndrome with muscle, eye and brain involvement. Acta Ophthalmol (Copenh) 56:465-472
28. Rosman NP, Kakulas BA (1966) Mental deficiency associated with muscular dystrophy: a neuropathological study. Brain 89:769-788
29. Santavuori P, Somer H, Sainio K, Rapola J, Kruus S, Nikitin T, Ketonen L, Leisti J (1989) Muscle-eye-brain disease (MEB). Brain Dev 11:147-153
30. Schröder JM, Bohl J, Brodda K (1978) Changes of the ratio between myelin thickness and axon diameter in the human developing sural nerve. Acta Neuropathol (Berl) 43:169-178
31. Serratrice G, Cros D, Pellissier J-F, Gastaut J-L, Pouget J (1980) Dystrophie musculaire congénitale. Rev Neurol (Paris) 136:445-472
32. Takada K, Nonaka I, Shioda K, Hasebe T, Takada C (1981) Two autopsied cases of Fukuyama type congenital muscular dystrophy, with particular reference to morphometric analysis of the anterior spinal roots (abstract). Brain Dev 3:165
33. Takada K, Nakamura H, Tanaka J (1984) Cortical dysplasia in congenital muscular dystrophy with central nervous system involvement (Fukuyama type). J Neuropathol Exp Neurol 43:395-407
34. Toda T, Segawa M, Nomura Y, Nonaka I, Masuda K, Ishihara T, Suzuki M, Tomita I, Origuchi Y, Ohno K, Misugi N, Sasaki Y, Takada K, Kawai M, Otani K, Murakami T, Saito K, Fukuyama Y, Shimizu T, Kanazawa I, Nakamura Y (1993) Localization of a gene for Fukuyama type congenital muscular dystrophy to chromosome 9q31-33. Nature Genet 5:283-286
35. Topaloğlu H, Yalaz K, Renda Y, Çağlar M, Göğüs S, Kale G, Gücüyener K, Nurlu G (1991) Occidental type cerebromuscular dystrophy: a report of eleven cases. J Neurol Neurosurg Psychiatry 54:226-229
36. Towfighi J, Sassani JW, Suzuki K, Ladda RL (1984) Cerebro-ocular dysplasia-muscular dystrophy (COD-MD) syndrome. Acta Neuropathol (Berl) 65: 110-123

37. Wargowski DS, Chitayat D, Tyson RW, Norman MG, Friedman JM (1991) Lethal congenital muscular dystrophy with cataracts and a minor brain anomaly: new entity or variant of Walker-Warburg syndrome? Am J Med Genet 39:19-24
38. Yoshioka M, Saiwai S (1988) Congenital muscular dystrophy (Fukuyama type): changes in the white matter low density on CT. Brain Dev 10:41-44
39. Yoshioka M, Okuno T, Ito M, Konishi Y, Itagaki Y, Sakamoto Y (1981) Congenital muscular dystrophy (Fukuyama type): repeated CT studies in 19 children. Comput Tomogr 5:81-88
40. Yoshioka M, Saiwai S, Kuroki S, Nigami H (1991) MR imaging of the brain in Fukuyama-type congenital muscular dystrophy. AJNR 12:63-65

Association of congenital muscular dystrophy, hypoplasia of the lateral abdominal wall musculature and hypoplasia of the external genitalia

Q.H. Leyten, W.O. Renier, F.J.M. Gabreëls,
H.G. Brunner, H.J. ter Laak, R.A. Mullaart

Neuropediatrics 1995, accepted for publication

Abstract

We describe a girl with the rare association of congenital muscular dystrophy, hypoplasia of the lateral abdominal wall musculature and hypoplasia of the external genitalia.

'Pure' congenital muscular dystrophy (CMD type I) is an autosomal recessive muscular disorder that is present at birth or manifests itself during the first year of life. It is characterized by generalized muscle weakness with a non-progressive or slowly progressive clinical course, (sub)normal cognitive development and muscle histology showing characteristic changes of fibre necrosis, fat cell infiltration and fibrous tissue infiltration [3, 10]. A subgroup of 'pure' CMD is associated with cerebral white matter hypodensity and merosin-negative staining of muscle biopsy specimens [12, 17, 18]. According to the new nomenclature, this condition is referred to as merosin M-chain-deficient or laminin-α_2-deficient CMD [1]. The laminin-α_2 gene is linked to chromosome 6q2 [4]. CMD can be associated with multiple joint contractures. Congenital joint contractures (arthrogryposis) also occur in amyoplasia [8]. Patients with amyoplasia have a higher frequency of non-limb anomalies, such as hypoplastic external genitalia [8] and defects in the lateral abdominal wall musculature [14].

We describe a girl with an association of CMD, hypoplasia of the lateral abdominal wall musculature and hypoplasia of the external genitalia.

Case Report

A girl, the first child of healthy non-consanguineous parents, was born after 40 weeks of gestation. There was no family history of neuromuscular disorders. Pregnancy was complicated by breech position and the infant was delivered by Caesarian section. Apgar score was 9 after 1 min. Birth weight was 3530 g, birth length 50 cm, occipitofrontal head circumference 36.5 cm (60^{th} centile). At birth, the neonate was hypotonic and weak and had contractures of the neck, thumbs, elbows and hips and had slight campylodactyly. Deep tendon reflexes were absent and pathological reflexes could not be elicited. There was striking hypoplasia of the lateral abdominal wall musculature (obliquus abdominis) with obvious diastasis (Fig. 1). The genitalia were female with absent labia majora. There was no webbing of the skin (pterygium).

On admission to hospital at 3 years of age, the child showed marked motor delay, generalized weakness, hypotonia, flexion contractures of the neck, thumbs, elbows and hips and slight campylodactyly. She was able to stand and walk, but only with support. Mental development was appropriate for her age. Examination of the eyes was normal.

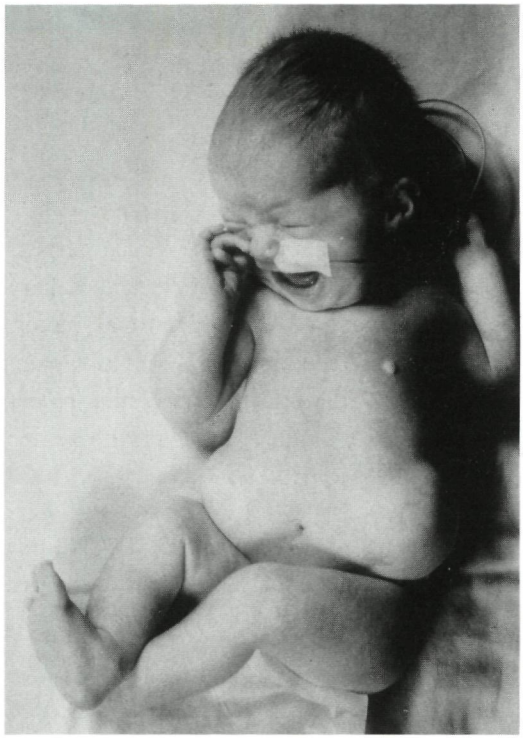

Fig. 1. The patient at 2 months of age with striking hypoplasia of the lateral abdominal wall musculature (obliquus abdominis) and obvious diastasis.

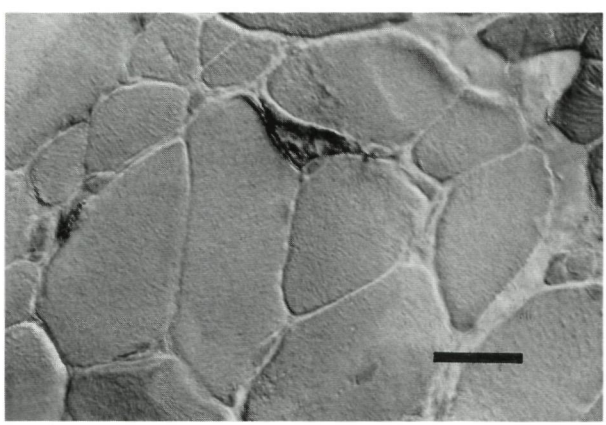

Fig. 2. Biopsy from the quadriceps muscle showing variation in fibre diameters, slight endomysial fibrosis and a dark staining regenerating fibre. Anti neonatal-myosin antibodies. Bar = 25 μm.

Investigations

The following investigations were normal full blood count, urea, creatinine, electrolytes, creatine kinase, carnitine, ammonia, proteins (including electrophoresis), lipids, ceruloplasmin, purines, pyrimidines, thyroid function, organic and amino acid levels in plasma and urine, white blood cell (lysosomal) enzymes, blood and cerebrospinal fluid (CSF) lactate and pyruvate and CSF analysis Serology for toxoplasma, rubella, cytomegalovirus, herpes and syphilis was negative Chromosome analysis revealed a normal 46,XX karyotype

Neurophysiological investigations revealed normal motor nerve conduction velocities of the peroneal and median nerves, but myopathic features, i e an increased number of brief, polyphasic, small amplitude potentials in the deltoid, quadriceps and tibialis anterior muscles consistent with myopathy There was no evidence of denervation/reinnervation

Magnetic resonance imaging (MRI) of the cerebrum, cerebellum, brain stem, medulla oblongata, spinal cord and cauda equina appeared normal MRI of the lateral abdominal wall musculature showed hypoplasia of the obliquus abdominis

Muscle biopsy

The needle biopsy obtained from the quadriceps muscle at the age of 22 months showed many type I fibres (67%) and wide variation in the fibre diameters caused by the presence of atrophic and hypertrophic fibres The muscle fibres were rounded and there was moderate perimysial fibrosis and extensive endomysial fibrosis (Fig 2) The number of fibres with internal nuclei was normal and slight fat cell infiltration was observed Some regenerating fibres (positive with LEU 19 antibodies and antibodies against neonatal and developmental myosin) were present (Fig 2) The muscle fibres showed normal dystrophin, vimentin, desmin and merosin (or laminin-α_2) staining Carnitine content was within the normal range Electron microscopic examination did not reveal any additional information

Discussion

Our patient's symptoms meet the clinical and morphological criteria for 'pure' congenital muscular dystrophy with normal mental development (CMD type I) [3, 10]. Her histopathological characteristics included extensive substitution of muscle tissue by endomysial fibrosis and some regenerating fibres. Necrosis was not observed, but it is usually less severe in CMD than in Duchenne dystrophy and limb girdle dystrophy, and it may even be absent in muscle specimens [5]. Fat cell infiltration was slight. This latter phenomenon has been found to increase with increasing age in 'pure' CMD [11] and usually differs from one site to another within one muscle [5]. The extracellular matrix protein laminin-α_2 was found to be present, which excludes the laminin-α_2-negative subgroup of 'pure' CMD with cerebral white matter hypodensity [12, 17, 18]. Laminin variants are proteins that are expressed in muscle membranes as well as in Schwann cells [1, 18]. The normal mental development and normal cerebral MRI in our patient are additional arguments in favour of 'pure' CMD type I.

Our patient with 'pure' CMD type I also had hypoplasia of the lateral abdominal wall musculature, as demonstrated by MRI, and hypoplasia of the external genitalia. Hypoplasia of the lateral abdominal wall musculature causes a prune belly aspect of the abdomen. The descriptive term 'prune belly' refers to the slack, lax abdomen alone and should be differentiated from the 'prune belly syndrome', a well-recognized entity consisting of deficient abdominal musculature, failure of testicular descent and structural urinary tract anomalies [6, 9, 13, 15, 16]. Although our patient had hypoplasia of the external genitalia, structural abnormalities of the urinary tract were lacking, which excluded a true 'prune belly syndrome' [6, 9, 11, 14, 15]. Our case seemed to bear some resemblance to cases with amyoplasia. In amyoplasia, limb muscles are absent and are replaced by fibrous and fatty tissue. Congenital joint contractures (arthrogryposis) have been described in amyoplasia by *Hall* et al [8]. At the same centre, *Reid* et al reviewed 225 cases of amyoplasia and observed other associated anomalies: 12 cases (5.3%) with gastroschisis, 6 (2.7%) with bowel atresia and 4 (1.8%) with defects in the lateral abdominal wall musculature [14].

Summarizing, our patient had characteristics of 'pure' CMD, a prune belly due to hypoplasia of the lateral abdominal wall musculature and hypoplasia of the external genitalia. In an attempt to classify our patient, there are three possible diagnoses: (1) a coincidental association of CMD and hypoplasia/aplasia of the lateral abdominal wall muscles, because of the epidemiological rarity of the two entities, (2) a non-coincidental association of CMD and hypoplasia/aplasia

of the lateral abdominal wall musculature and (3) a 'new' CMD subtype with prominent involvement of the lateral abdominal wall muscles, that presents with a prune belly in the neonatal period. We are presently unable to differentiate between these three diagnoses. The first hypothesis receives some indirect support from observations in hereditary aplasia of specific muscles [2, 7]. The second hypothesis is in line with amyoplasia, in which lateral wall hypoplasia is commonly seen [8, 14]. Molecular genetic investigations may eventually reveal which of these hypotheses is correct.

References

[1] *Burgeson, R.E., M. Chiquet, R. Deutzmann, P. Ekblom, J. Engel, H. Kleinman, G.R. Martin, G. Meneguzzi, M. Paulsson, J. Sanes,* et al: A new nomenclature for the laminins. Matrix Biol. 14 (1994) 209-211

[2] *David, T.J., R.M. Winter:* Familial absence of the pectoralis major, serratus anterior, and latissimus dorsi muscles. J. Med. Genet. 22 (1985) 390-392

[3] *Dubowitz, V.:* Workshop report: 22nd ENMC sponsored workshop on congenital muscular dystrophy held in Baarn, The Netherlands, 14-16 May 1993. Neuromusc. Disord. 4 (1994) 75-81

[4] *Dubowitz, V., M. Fardeau:* Workshop report: proceedings of the 27th ENMC sponsored workshop on congenital muscular dystrophy. 22-24 April 1994, The Netherlands. Neuromusc. Disord. 5 (1995) 253-258

[5] *Emery, A.H.:* Diagnostic criteria for neuromuscular disorders. European Neuromuscular Centre (1994)

[6] *Greskovich, F.J., L.M. Nyberg:* The prune belly syndrome: A review of its etiology, defects, treatment and prognosis. J. Urol. 140 (1988) 707-712

[7] *Gross-Kieselstein, E., R.S. Shalev:* Familial absence of the trapezius muscle with associated shoulder girdle abnormalities. Clin. Genet. 32 (1987) 145-147

[8] *Hall, J.G., S.D. Reed, E.P. Driscoll:* Part I. Amyoplasia: A common, sporadic condition with congenital contractures. Am. J. Med. Genet. 15 (1983) 571-590

[9] *Hoagland, M.H., G.M. Hutchins:* Obstructive lesions of the lower urinary tract in the prune belly syndrome. Arch. Pathol. Lab. Med. 111 (1987) 154-156

[10] *Leyten, Q.H., F.J.M. Gabreëls, W.O. Renier, H.J. ter Laak, R.C.A. Sengers, R.A. Mullaart:* Congenital muscular dystrophy. J. Pediatr. 115 (1989) 214-221

[11] *Leyten, Q.H., H.J. ter Laak, F.J.M. Gabreëls, W.O. Renier, R.C.A. Sengers:* Congenital muscular dystrophy: A study on the variability of morphological changes and dystrophin distribution in muscle biopsies. Acta Neuropathol. 86 (1993) 386-392

[12] *Mercuri, E., F. Muntoni, A. Berardinelli, J. Pennock, C. Sewry, J. Philpot, V. Dubowitz:* Somatosensory and visual evoked potentials in congenital muscular dystrophy: Correlation with MRI changes and muscle merosin status. Neuropediatrics 26 (1995) 3-7

[13] *Nakayama, D.K., M.R. Harrison, D.H. Chinn, A.A. De Lorimier:* The pathogenesis of prune belly. Am. J. Dis. Child. 138 (1984) 834-836

[14] *Reid, C.O.M.V., J.G. Hall, C. Anderson, M. Bocian, J. Carey, T. Costa,* et al: Association of amyoplasia with gastroschisis, bowel atresia and defects of the muscular layer of the trunk. Am. J. Med. Genet. 24 (1986) 701-710

[15] *Reinberg, Y., E. Shapiro, J.G. Manivel, C.B. Manley, G. Pettinato, R. Gonzalez:* Prune belly syndrome in females: A triad of abdominal musculature deficiency and anomalies of the urinary and genital systems. J. Pediatr. 118 (1991) 395-398

[16] *Smeitink, J., B.C.J. Hamel, R. van Empelen, J.D.M. de Vries, L.A.H. Monnens:* The prune belly syndrome: Experiences in nine patients. Ned. Tijdschr. Geneeskd. 131 (1987) 489-492

[17] *Tomé, F.M.S., T. Evangelista, A. Leclerc, Y. Sunada, E. Manole, B. Estournet, A. Barois, K.P. Campbell, M. Fardeau:* Congenital muscular dystrophy with merosin deficiency. C. R. Acad. Sci. Paris 317 (1994) 351-357

[18] *Voit, T., C.A. Sewry, K. Meyer, R. Hermann, V. Straub, F. Muntoni, T. Kahn, R. Unsöld, T.R. Helliwell, R. Appleton, H.G. Lenard:* Preserved merosin M-chain (or laminin-α_2) expression in skeletal muscle distinguishes Walker-Warburg syndrome from Fukuyama muscular dystrophy and merosin-deficient congenital muscular dystrophy. Neuropediatrics 26 (1995) 148-155

LOCALIZED FORMS OF CONGENITAL MUSCULAR DYSTROPHY

Dystrophic myopathy of the diaphragm in a neonate with severe respiratory failure during infectious episodes

Q.H. Leyten, W.O. Renier, F.J.M. Gabreëls,
H.J. ter Laak, L.H.A. Hinkofer

Neuromuscular Disorders 1993;3:51-55

Abstract

In this study a boy is described who showed slight postnatal asphyxia related to isolated dystrophic diaphragmatic musculature. Development was complicated by several periods of bronchopneumonia necessitating artificial respiration each time.

Very few cases of respiratory insufficiency due to dystrophic diaphragmatic musculature have been reported [1-4]. Only two cases have been described with isolated dystrophy of the diaphragmatic musculature: in one case a 'dystrophy-like' diaphragm was found [4] and in another well-documented case isolated dystrophy of diaphragmatic musculature was seen [2]. In this paper, we report the clinical and morphological characteristics of another case with isolated dystrophic diaphragmatic musculature which led to severe asphyxia in periods of bronchopneumonia necessitating artificial respiration.

Case Report

A boy, the second child of healthy non-consanguineous parents, was born at 37 weeks. Pregnancy was complicated by slight toxicosis, decreased antenatal movements and breech position, and was terminated by caesarian section. Apgar score was 8 after 5 min. Birth weight was 2,750 g. Occipitofrontal circumference was 33.5 cm. The postpartum period was complicated by a short period of hypoxia and slight generalized hypotonia. Examination revealed upwards displacement of the right part of the diaphragm. No muscular atrophy, muscular weakness, talipes, tent-shaped mouth and no haematomas were observed. Deep tendon reflexes were present, pathological reflexes could not be elicited. Slight resistance to passive movements of the hips and elbows was noticed. Mild retrognathia and asymmetrical implant of the ears were seen.

The family history did not mention neuromuscular diseases, such as dystrophic myotonia, myasthenia or congenital myopathies. Further development of the boy was complicated by periods of bronchopneumonia caused by *Staphylococcus aureus* and *Haemophilus influenzae*, respectively, which led to severe asphyxia and febrile convulsions, necessitating artificial respiration for 2-3 days. Despite five periods of artificial respiration, the motor and mental development have proceeded normally so far. At the time of this study, he was 3 yr old. To correct the upwards displacement of the right diaphragm, restorative surgery was undertaken at the age of 19 months. At the operation, eventration was present. The diaphragm was hypotonic with a normal macroscopic aspect. A biopsy specimen was taken from the right diaphragm. Further investigations on the respiratory tract and cardiovascular system did not reveal any other abnormalities.

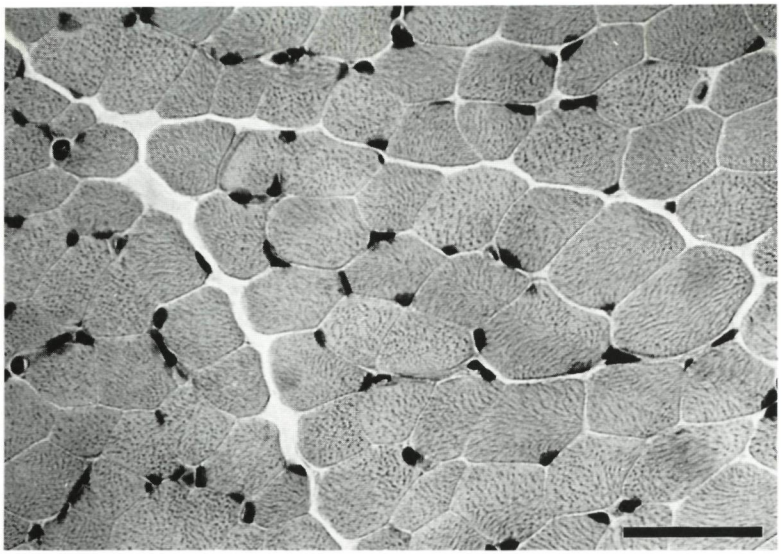

Fig. 1. Muscle biopsy from quadriceps muscle at the age of 14 months showing a normal picture (HE, bar = 50 μm).

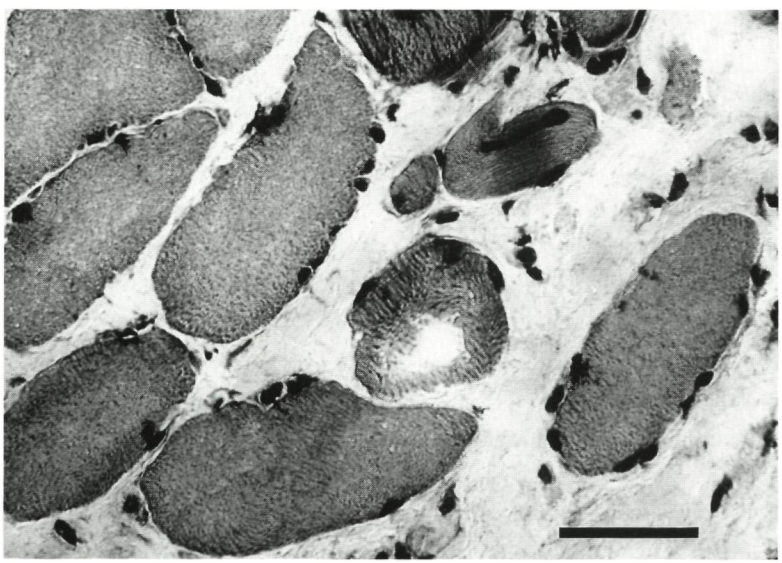

Fig. 2. Muscle biopsy from diaphragm muscle showing many rounded hypertrophic fibres with a clear increase in endomysial connective tissue (HE, bar = 50 μm).

Laboratory studies

Results of the following laboratory tests were normal: complete blood cell count and urine analysis, renal function tests, determinations of serum electrolyte levels, serum protein content and electrophoretic pattern, serum lipid spectrum, ceruloplasmin, ammonia, purines, pyrimidines, organic and amino acid levels in plasma and urine, and lysosomal enzyme activities in leucocytes. Appropriate studies ruled out endocrinological diseases, especially hypothyroidism and disorders caused by toxic agents. Lactate and pyruvate levels were normal in serum and the cerebrospinal fluid (CSF). Further studies on the CSF showed no abnormalities; leucocyte count, protein immunoelectrophoresis and ketone bodies were normal. Creatine kinase (CK) activity was normal (27 U l^{-1}). Myoglobin and carnitine content were normal.

Microbiological examination excluded infections caused by toxoplasma, cytomegalovirus, herpes and rubella virus, and syphilis.

Electromyographic studies revealed normal motor and sensory nerve conduction velocities in the arms and legs, and normal myographic patterns. Stimulation of the right phrenic nerve was not attempted. Prostigmine test was normal.

Muscle biopsies

Needle biopsies from the quadriceps muscle (17 days and 14 months after birth) did not reveal any dystrophic characteristics (Fig. 1). Immunohistochemical localization of dystrophin, desmin and vimentin appeared to be normal.

Contrary to the results of both biopsies from the quadriceps muscle, a muscle specimen from the diaphragm taken at 19 months of age, clearly showed a dystrophic aspect by the presence of many rounded hypertrophic muscle fibres embedded in a matrix of connective tissue (Fig. 2). Sporadically, a muscle fibre with a round vacuole filled with a basophilic substance was observed. The number of muscle fibres with internal nuclei was increased; fat cells and fibre splitting were absent. Enzyme histochemically, most of the fibres were type I fibres; some of the fibres showed gradations in staining intensity with ATPase (preincubated at pH 4.2) and thus resembled type IIC fibres. The 5'-nucleotidase activity of the connective tissue was clearly increased. Immunohistochemical localization of dystrophin, desmin and vimentin appeared to be normal. Ultramicroscopic examination of the same specimen did not reveal any other abnormalities. Myoneural endplates were normal. Pyruvate oxidation, fatty acid oxidation and carnitine content of muscle tissue were within the normal ranges.

Discussion

The boy in our study suffered from severe respiratory insufficiency, but only during infectious episodes. Obstructive upper airway pathology was excluded. The sole anomaly was isolated dystrophy of the diaphragmatic musculature. Despite five periods of artificial respiration, the boy is developing well.

Neurological causes of diaphragmatic paralysis were excluded, such as pathology of the anterior horn (poliomyelitis, carcinomatous encephalomyelitis, amyotrophic lateral sclerosis, chronic proximal spinal muscular atrophy, Werdnig-Hoffmann disease [5-7]), or the roots of the cervical plexus (traumatic, tuberculosis, cervical disc hernia, cervical spine spondylarthrosis, birth injuries with Erb's palsy). Disturbances of the peripheral nerves (the Guillain-Barré syndrome, blastomatosis, phrenic nerve trauma [8] and neuritis by infections such as poliomyelitis, diphtheria, syphilis and herpes zoster, were excluded, as well as disturbances caused by toxic (lead), metabolic (acute intermittent porphyria, nutritional beriberi), allergic (vascular) and endocrinological agents (congenital hypothyroidism [9]). Muscle diseases such as primary infection of the diaphragm [10,11] by cytomegalovirus, neonatal myotonic dystrophy [12-16], myasthenia gravis and polymyositis [17] were also excluded.

With respect to the histological characteristics of the muscle biopsies taken from our patient, it should be emphasized that no changes were found in the quadriceps muscles. The diaphragmatic musculature revealed typical dystrophic changes, such as an increased variation in the fibre diameter, an increased number of fibres with internal nuclei, and rounded muscle fibres surrounded by increased quantities of collagen. Immature, myotube-like fibres as seen in Werdnig-Hoffmann disease [18] were not observed. Myotubes (very small fibres with swollen internal nuclei), as seen in muscles (including the diaphragm) from cases suffering from congenital myotonic dystrophy [16], were not present in the diaphragm of our case.

Reports in the literature on respiratory insufficiency due to (isolated) dystrophy of the diaphragmatic musculature are scarce [1-4] and most of them are poorly documented [2]. Only Bergen *et al.* presented a well-documented case of a myopathic process in a neonate which solely involved the diaphragm [2]. In their study, isolated diaphragmatic dystrophy was found with typical dystrophic characteristics, such as an increased variation in the fibre diameter, increased amount of collagen, necrosis and hypertrophy. All the other muscles examined (psoas, deltoid, biceps, quadriceps, gastrocnemius, intercostal, neck and tongue muscles) were normal. The female infant in their study died at 3 months of age.

They emphasized that possible 'hypoplasia' or 'dysplasia' of the diaphragm was very unlikely, because the intercostal and abdominal muscles, which originate from the same myotomes as the diaphragm, did not reveal any similar changes.

Bosman *et al.* [4] described a case of congenital diaphragmatic paralysis in an infant who died of respiratory failure at the age of 5 weeks. At autopsy they found dystrophy-like muscle pathology restricted to the diaphragm. The histological findings included extensive thinning of the diaphragm, marked variation in muscle fibre size with random distribution, nuclei in rows in the more atrophic fibres and increased interstitial tissue, but without inflammatory infiltrates, fatty or fibrotic changes. They hypothesized hypoplasia of the diaphragm muscle, leading to compensatory hypertrophy of the remaining muscle fibres, consequently determining a muscular dystrophy-like appearance of the diaphragm, with random distribution of large and small fibres. The female infant in their study died at the age of 1 month and 7 days.

Lewis and Besant [1] reported two patients, a boy and a girl, in a family with two siblings with diaphragmatic weakness. The histological findings in the diaphragm did not reveal an increased amount of interstitial tissue. The variation in muscle fibre size was slight with respect to our case. The other striate muscles examined showed no changes, except for the pectoral muscle in one case, which also showed slightly increased variation in the size of the fibres and a rare vacuolated fibre. The boy died at the age of 8 weeks, the girl at the age of 6 weeks.

De Reuck *et al.* [3] described a patient with progressive congenital myopathy, with initial involvement of the diaphragm with type I muscle fibre atrophy, but also involving the peripheral psoas and pectoralis major muscles. The histological characteristics of the diaphragm included an increased amount of collagen, increased variation in the fibre diameter, but no hypertrophy. Fibres with internal nuclei were not observed. The boy in the study by De Reuck *et al.* [3] died at the age of 21 months.

Our study of the literature led us to the conclusion that only Bergen *et al.* [2] described a case with isolated dystrophy of the diaphragm. Based on the histological data, we believe that our patient represents a second well-documented case. An important clinical difference is the clinical course. The female infant in Bergen's study died at 3 months of age, in contrast to our male patient who is still alive at the age of 3 yr. So far, our patient has had five periods of bronchopneumonia necessitating artificial respiration during each period. Whether our patient represents a new (variant) type of diaphragmatic muscular dystrophy with a milder clinical course, remains unclear. Despite the life-threatening events, he is still developing well.

References

1. Lewis A J, Besant D F. Muscular dystrophy in infancy: report of 2 cases in siblings with diaphragmatic weakness. *J Pediatr* 1962;**60**:376-384.
2. Bergen B J, Sangalang V E, Aterman K. Isolated myopathic involvement of the diaphragmatic musculature in a neonate. *Ann Neurol* 1977;**1**:403-407.
3. De Reuck J, Hooft C, De Coster W, Van den Bossche H, Cuvelier C. A progressive congenital myopathy: initial involvement of the diaphragm with type I muscle fiber atrophy. *Eur Neurol* 1977;**15**:217-226.
4. Bosman C, Bachelet V, Boldrini R, Bertini E. Diaphragmatic paralysis due to partial diaphragmatic hypoplasia mimicking a localized muscular dystrophy: a case report. *Clin Neuropathol* 1988;**7**:33-38.
5. Mellins R B, Hays A P, Gold A P, *et al.* Respiratory distress as the initial manifestation of Werdnig-Hoffmann's disease. *Pediatrics* 1974;**53**:33-41.
6. Meunier-Carus J, Lonsdorfer J, Lampert E. Répercussions functionelles cardio-respiratoires des atteintes neuro-musculaires de la paroi thoraco-abdominale. *Poumon Coeur* 1974;**30**:13-19.
7. Parhad I M, Clark A W, Barron K D, Staunton S B. Diaphragmatic paralysis in motor neuron disease: report of two cases and a review of the literature. *Neurology* 1978;**28**:18-22.
8. Larson R K, Evans B H. Eventration of the diaphragm. *Am Rev Respir Dis* 1963;**87**:753-756.
9. Pollard B J, Hain W R, Small P G. Paralysis of the right hemidiaphragm. *Anaesthesia* 1982;**37**:195-200.
10. Wayne E R, Burrington J D, Myers D N, *et al.* Letter: Bilateral eventration of the diaphragm in a neonate with congenital cytomegalic inclusion disease. *J Pediatr* 1973;**83**:164-165.
11. Becroft D M. Prenatal cytomegalovirus infection and muscular deficiency (eventration) of the diaphragm. *J Pediatr* 1979;**94**:74-75.
12. Bossen E H, Shelburne J D, Verkauf B S. Respiratory muscle involvement in infantile myotonic dystrophy. *Arch Pathol* 1974;**97**:250-252.
13. Kilburn K H, Eagan J T, Sieker H O, Heyman A. Cardiopulmonary insufficiency in myotonic and progressive muscular dystrophy. *N Engl J Med* 1959;**261**:1089-1096.
14. Aicard J, Conti D, Goutières F. Les formes néo-natales de la dystrophie myotonique de Steinert. *J Neurol Sci* 1974;**22**:149-164.

15. Billeaud C, Baronnet R, Cadier L, Coquet Mme, Demarquez J L, Martin C. Détresse respiratoire chez le nouveau-né atteint de maladie de Steinert. *La Médicine Infantile* 1982;**89**:35-49.
16. Sarnat H B, Silbert S W. Maturational arrest of fetal muscle in neonatal myotonic dystrophy: a pathologic study of four cases. *Arch Neurol* 1976; **33**:466-474.
17. De Reuck J, De Coster W, Inderadjaja N. Acute viral polymyositis with predominant diaphragm involvement. *J Neurol Sci* 1977;**33**:453-460.
18. Hausmanowa-Petrusewicz I, Fidzianska A. Spinal muscular atrophy: foetal-like histopathological pattern in Werdnig-Hoffmann disease. *Bull NY Acad Med* 1974;**50**:1157-1172.

LATE ONSET TYPE OF MUSCULAR DYSTROPHY

Familial adult-onset muscular dystrophy with leukoencephalopathy

B.G.M. van Engelen, Q.H. Leyten, P.L.J.A. Bernsen, F.J.M. Gabreëls,
F. Barkhof, E.M.G. Joosten, B.C.J. Hamel, H.J. ter Laak,
M.B.M. Ruijs, J.R.M. Cruysberg, J. Valk

Annals of Neurology 1992;32:557-580

Abstract

We report on 3 siblings with an adult-onset, predominantly distal muscle weakness. In the female index patient this was associated with epilepsy and a progressive spastic ataxic gait, while the 2 other siblings had no appreciable clinical nervous system involvement. Additional investigations revealed muscular dystrophy and leukoencephalopathy in all 3 siblings. We conclude that this familial adult-onset muscular dystrophy associated with leukoencephalopathy represents a newly recognized autosomal recessive syndrome.

An association between muscular dystrophy and leukoencephalopathy has been described in congenital muscular dystrophy (CMD) of the Fukuyama type (F-CMD); CMD of the so-called and less severe non-Fukuyama type (nF-CMD) or "Occidental type"; and the composite "muscle, eye, and brain disease" (MEB) [1-4]. Central nervous system (CNS) involvement may range from apparently asymptomatic leukoencephalopathy (nF-CMD) to widespread developmental abnormalities in the cerebral architecture (F-CMD, MEB). These three types of CMD appear immediately after birth and are always connected with severely delayed motor development.

We present the cases of 3 siblings with a type of muscular dystrophy combined with leukoencephalopathy in whom symptoms presented in adulthood. The relationship with CMD is discussed.

Case Histories

Patient III-3 (proband)

The proband (Fig. 1) was a 29-year-old woman who had normal motor milestones and intellectual development until the age of 18 years. Then she experienced exercise-induced walking difficulties due to a mainly left-sided footdrop. At the age of 22, she had her first generalized tonic-clonic seizure. Afterward, walking difficulties due to muscle weakness in the lower part of the legs and spastic gait were found. Following each epileptic attack, her walking deteriorated further. At the age of 28 years, she became wheelchair dependent. Neurological examination showed bilateral myopia (– 2.0 diopters), slightly increased calf bulk, and bilateral pes cavus without muscle wasting. There was a diffuse weakness of all muscle groups that was more pronounced distally in the lower limbs. She had a bilateral predominantly right-sided pyramidal and cerebellar syndrome. Cognitive functions were normal.

Patient III-4 (affected sib)

This 25-year-old man has always been healthy. He admitted to having some exercise-induced pain in the upper leg muscles and calves. Neurological examinations showed bilateral myopia (– 0.75 diopters), an increased calf bulk, and slight distal muscle weakness in the lower extremities.

Patient III-5 (affected sib)

This 19-year-old man started to complain of abnormal muscle fatigue after exercise from the age of 16 years. At 18 years old, he sought medical attention because of slight exercise-induced muscle weakness and pain in the upper leg muscles and calves. Neurological examination revealed bilateral myopia (– 7.5 diopters), increased calf bulk, pes cavus, and slight distal muscle weakness in the lower extremities.

Family history

The medical history and examination of Patients III-2, II-3, II-9, I-1, I-3, I-4, II-1, II-2, II-4, II-5, II-6, II-7, II-10, IV-1, IV-2, and IV-3 did not reveal any abnormalities. Pedigree analysis revealed no consanguinity in six generations.

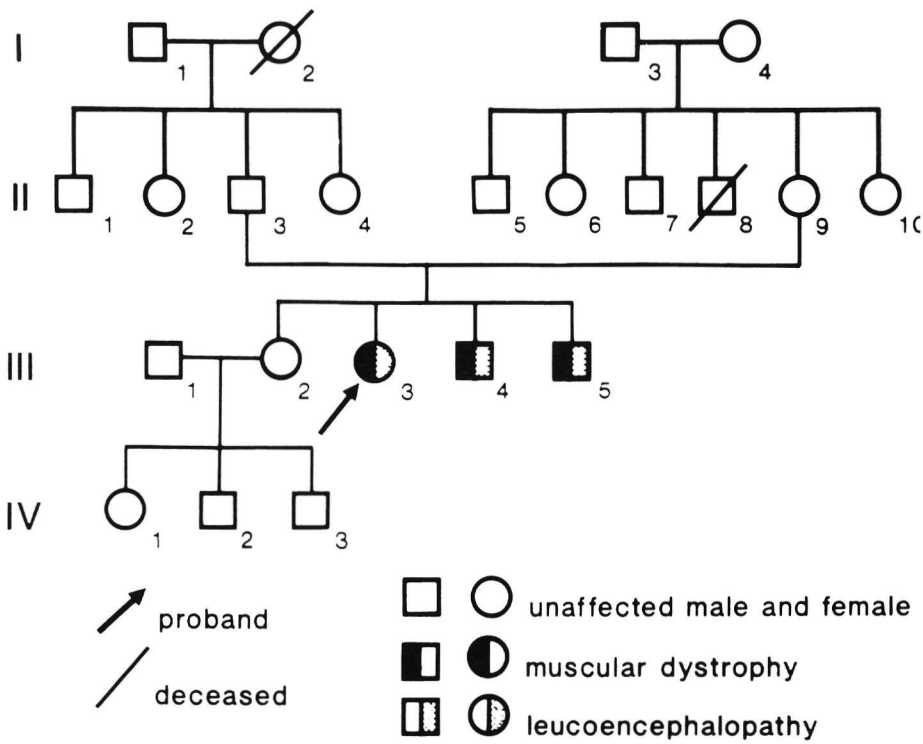

Fig. 1. Pedigree of the present family. Affected family members were Patients III-3, III-4, and III-5.

Table. Summary of investigations in affected siblings

Tests	Patient III-3	Patient III-4	Patient III-5
Laboratory			
Creatine kinase (units/liter; normal < 90)	116-281	586-801	791-1608
Dystrophy gene rearrangements	NE	NE	−
Muscle biopsy (dystrophic changes)	+	NE	+ +
Neurophysiology			
Electromyography (myopathic)	+	+ +	+ + +
Nerve conduction velocity slowing	−	−	−
Brainstem auditory evoked potential slowing	+ +	− +	−
Somatosensory evoked potential slowing	+ +	−	−
Visual evoked potential slowing	−	−	−
Electroencephalography			
Slowing	+ +	+	+ +
Slow spike waves	+	−	+ +
Neuroradiology			
Brain CT scan: white matter hypodensities	+ + +	+	+ +
Brain MRI			
Periventricular hyperintensities	+ +	+	+
Deep white matter hyperintensities	+ + +	+	+ +
U fiber involvement	+ + +	−	+ +
Corpus callosum atrophy	+ +	−	+
External capsule involvement	+ +	+	+ +
CT scan of muscles: fatty infiltrates	−	+	+ +

− = absent; − + = borderline; + = mild; + + = moderate; + + + = severe; NE = not estimated.

Results

Laboratory investigations

Creatine kinase values were clearly elevated and varied among the 3 affected siblings (Table). Unaffected family members (II-3, II-9, III-2, IV-1, IV-2, IV-3) had normal CK values. Results of the following laboratory tests were normal in Patients III-3 and III-5: in urine––organic acids, amino acids, purines, pyrimidines, mucopolysaccharides, oligosaccharides, and neuraminic acid; in serum ––amino acids, very-long-chain fatty acids, and lactate; in leukocytes––several lysosomal enzymes (including acid maltase, arylsulfatase A, galactocerebrosidase); and in cerebrospinal fluid––total protein, IgG, and lactate. Ischemic forearm exercise tests revealed normal findings. Acanthocytes were not found. Rearrangements of the dystrophy gene at Xp21 were not found in Patient III-5 [5].

Neurophysiological and ophthalmological studies

Electromyography (EMG) showed small polyphasic motor units, high-frequency discharges, fibrillations, and positive spikes mainly in the distal musculature in all 3 affected siblings (Table). Unaffected family (II-3, II-9, III-2) had normal EMG findings. Varying degrees of electroencephalographic (EEG) abnormalities were detected in all 3 affected siblings and consisted of diffuse slowing, focal hypofunctional, and irritative activity and generalized slow spike waves.

Corrected visual acuity was 20/20 in all 3 siblings. Slit-lamp microscopy and ophthalmoscopy revealed no abnormalities, especially no cataracts. Results of color vision tests were normal and the electroretinogram was not disturbed.

Radiological studies in brain and muscle

Leukoencephalopathy was defined as confluent, not well-demarcated areas of high-signal intensity on long repetition time (TR; 2,500 msec), long echo time (TE; 60 msec), spin-echo magnetic resonance images (MRIs) (0.6 tesla), corresponding with areas of low attenuation on the computed tomogram (CT). Leukoencephalopathy with mild ventricular enlargement was present in the proband (III-3, Fig. 2A) and to a lesser degree in both other affected siblings (III-4, III-5) (Table), whereas brain MRI showed no abnormalities in the asymptomatic sister (III-2) and in both parents (II-3, II-9). No progression of white matter abnormalities in Patient III-3 was seen on CT during the past 7 years. Short TR, spin-echo images were obtained after the injection of gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) in Patients III-3 and III-4, but no signal

enhancement was seen. There were no gyral disorders or calcifications, and no abnormalities of the anterior commissure, the internal capsule, the basal ganglia, the brainstem, or the cerebellum.

Fatty infiltrates were seen on the CT scan of the gastrocnemius and soleus muscles and to a lesser extent in the quadriceps muscles of Patients III-4 and III-5.

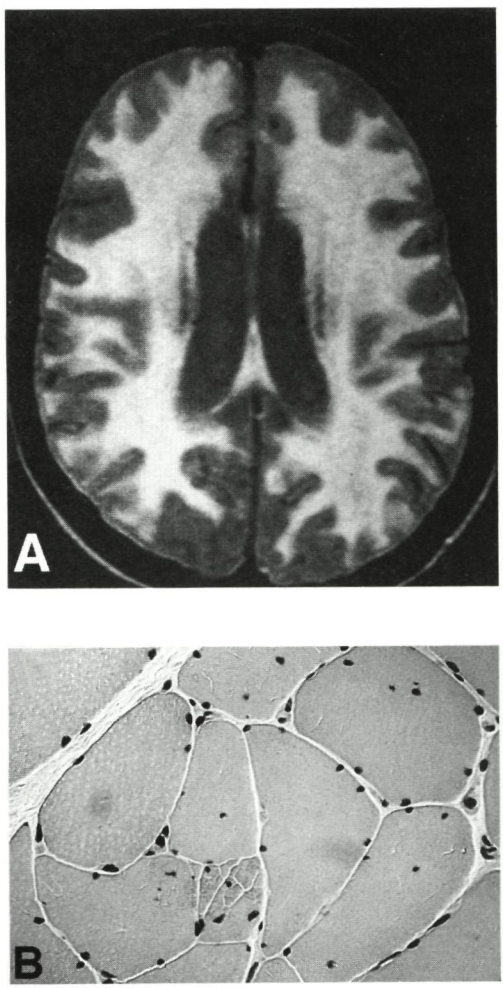

Fig. 2. (A) Increasing signal intensity of all the supratentorial white matter on MRI of the brain of Patient III-3. (B) Soleus muscle biopsy from Patient III-3 showing fiber splitting and fragmentation, an increased number of fibers with internal nuclei, and slight endomysial fibrosis.

Histopathological studies

Histopathological studies on frozen sections (quadriceps muscle) from Patients III-3 and III-5 showed characteristic findings of muscular dystrophy (Fig. 2B): increased variation of the muscle fiber diameter, fiber splitting and fragmentation, atrophic basophilic fibers, increased number of fibers with internal nuclei, replacing fat cells, and slight endomysial fibrosis. Enzyme histochemical studies on frozen sections including succinate dehydrogenase and cytochrome oxidase were normal. No ragged red fibers and no basophilic and rimmed vacuoles were found. In Patient III-3, a combined biopsy specimen of the soleus muscle and sural nerve showed more pronounced dystrophic changes in the soleus as compared to the quadriceps muscle. Morphological and morphometrical analysis of the sural nerve revealed no abnormalities using standard techniques [6].

In Patient III-5, immunostaining with monoclonal antibodies against the C-terminus of dystrophin (DYS 2, Novocastra Laboratories, Newcastle-upon-Tyne, UK) showed a normal pattern.

Biochemical studies in muscle

Substrate oxidation and ATP production studies were performed in 600-gm supernatants of the fresh muscle material obtained from the quadriceps. These studies, and the measurement of the activities of citrate synthase, cytochrome *c* oxidase, NADH:Q1 oxidoreductase, pyruvate dehydrogenase complex, and succinate:cytochrome *c* oxidoreductase and of the concentrations of carnitine and protein were performed as previously described [7]. A defect in oxidative metabolism was not found.

Discussion

The clinical features and results of additional investigations in our 3 patients do not correspond with any known neuromuscular syndrome in adulthood. Becker's muscular dystrophy is clinically unlikely because of the predominance of distal muscle weakness, the only moderately elevated CK levels, and the leukoencephalopathy. It is also genetically unlikely because of the female proband, the lack of rearrangement of the Xp21 gene, and the normal dystrophin level. Our patients could be compared to the type of distal muscular dystrophy described by Miyoshi and colleagues [8]. In the present family, however, the clinical findings included a more generalized form of muscular dystrophy, an only moderately elevated CK level, and no or only slight progression. In addition, no

association has been described between distal muscular dystrophy and leukoencephalopathy.

Leukoencephalopathies could, in our patients, correspond with either demyelination or dysmyelination due to developmental disturbances. Fluid accumulation in the white matter without demyelination or dysmyelination seems unlikely because of the lack of signs of brain swelling and the presence of mild ventricular enlargement. Known demyelinating leukoencephalopathies, including those due to mitochondrial diseases, were excluded on clinical and biochemical grounds. The lack of progression of leukoencephalopathy on successive CT scans suggests a dysmyelinating condition.

Recently, the familial occurrence of asymptomatic periventricular leukoencephalopathy with myopathy starting in early childhood was reported as a new syndrome [9]. In this myopathy, changes resembling inclusion body myositis were observed. In biopsy specimens from our patients, no basophilic vacuoles, an indication of inclusion bodies, were observed.

The clinical picture and associated CNS abnormalities of CMD show a marked heterogeneity, but no doubt exists about the congenital character of CMD [1]. "Adult-onset CMD" has not been hypothesized in the literature. CMD, nevertheless, is the only clinical syndrome comparable to that of the reported patients. Their clinical features and MRI findings do, however, not correspond to the severe neuromorphological alterations and functional or structural eye changes known to occur in F-CMD and MEB. The syndrome in the reported family shows remarkable similarities with, and might therefore possibly be, an adult-onset variant of nF-CMD. In the latter syndrome there is no correlation between the extent of leukoencephalopathy and the clinical signs of CNS involvement or the severity of the myopathy. nF-CMD may even present with asymptomatic leukoencephalopathy. Similar findings have been demonstrated in our patients, although it must be stated that leukoencephalopathy is most pronounced in the only sibling with clinically evident CNS involvement.

Similar to CMD, the mode of inheritance in the present family is most likely autosomal recessive with variable expression. The occurrence in siblings of both sexes from normal parents is sufficient evidence of an autosomal recessive trait.

We conclude that there is a type of muscular dystrophy associated with leukoencephalopathy which starts in adulthood and represents a newly recognized autosomal recessive syndrome. Provided CMD is not heterogenetic, the reported syndrome might be due to allelic mutation at the putative CMD locus.

References

1. Banker BQ. Congenital muscular dystrophy. In: Engel AG, Banker BQ, eds. Myology, vol 2. New York: McGraw-Hill, 1986:1367-1382
2. Topaloglu H, Yalaz K, Renda Y, et al. Occidental type cerebromuscular dystrophy: a report of eleven cases. J Neurol Neurosurg Psychiatry 1991;54:226-229
3. Trevisan CP, Carollo C, Segalla P, et al. Congenital muscular dystrophy: brain alterations in an unselected series of Western patients. J Neurol Neurosurg Psychiatry 1991;54:330-334
4. Leyten QH, Gabreëls FJM, Renier WO, et al. Congenital muscular dystrophy. J Pediatr 1989;115:214-221
5. Den Dunnen JT, Grootscholten PM, Bakker E, et al. Topography of the Duchenne muscular dystrophy (DMD) gene: FIGE and cDNA analysis of 194 cases reveals 115 deletions and 13 duplications. Am J Hum Genet 1989;45:835-847
6. Vos AJM, Joosten EMG, Gabreëls-Festen AAWM. Adult polyglucosan body disease: clinical and nerve biopsy findings in two cases. Ann Neurol 1983;13:440-444
7. Fischer JC, Ruitenbeek W, Gabreëls FJM, et al. A mitochondrial encephalomyopathy: the first case with an established defect at the level of coenzyme Q. Eur J Pediatr 1986;144:441-444
8. Miyoshi K, Kawai H, Iwasa M, et al. Autosomal recessive distal muscular dystrophy as a new type of progressive muscular dystrophy; seventeen cases in eight families including an autopsied case. Brain 1986;109:31-54
9. Cole AJ, Kuzniecky R, Karpati G, et al. Familial myopathy with changes resembling inclusion body myositis and periventricular leucoencephalopathy, a new syndrome. Brain 1988;111:1025-1037

Chapter V

GENERAL DISCUSSION

This study opens with a review of the literature and presents the clinico-pathological results in our own material on 34 patients with the diagnosis of congenital muscular dystrophy (CMD). Nearly 1% of all the patients who underwent a muscle biopsy at the Centre for the Investigation of Disorders of the Neuromuscular System at the University of Nijmegen in the period 1977-1994 had CMD. We discuss our findings in the light of the new classification for CMD as proposed at two recent international workshops on this topic, in which the author of this thesis was one of the participants.[1,2]

CMD type IA. Classical or 'pure' CMD without white matter hypodensity

The clinico-pathological observations in our patients[3-7] were all in accordance with those reported in the literature.[1,2,8-24]

CMD type IB. Classical or 'pure' CMD with white matter hypodensity

In seventeen patients with classical or 'pure' CMD, the neuroradiological examination (CT/MRI) showed three with hypodense white matter areas.[4] Two out of these three patients had epilepsy, but none were mentally subnormal. In the literature, the occurrence of epilepsy was mentioned in 13 out of 64 patients with a combination of CMD, (sub)normal intelligence and hypodense white matter areas.[1,25-37] Based on these data, it can be concluded that the frequency of epilepsy in CMD patients with (sub)normal intelligence and with white matter hypodensities is significantly higher (1 : 4.5) than in the normal population (1 : 150). The nature and significance of the white matter changes remain obscure. Informative neuropathological data are scarce. Egger et al[27] found patchy demyelination of the white matter of the centrum semiovale; Echenne et al[28] described a spongy appearance of the white matter on necropsy; and Malik et al[38] mentioned a case with significantly decreased myelin staining. The lack of progression of white matter hypodensities on successive CT/MRI scans[4,27,32,34,39] and normal myelin basic protein findings in CSF[4] are arguments in favour of hypo- or dysmyelination. Recently, Voit et al[40] postulated that the white matter hypodensities on CT/MRI are due to dysmyelination of the central nervous system. However, long-term follow-up studies with neuropathological, biochemical and magnetic resonance spectrometry (MRS) investigations are necessary to provide a definite answer to this question.

Severely decreased merosin expression was also observed in two of our patients with white matter changes in whom merosin investigation was possible. Until now, no direct relation has been demonstrated between hypodensities in the white matter and a merosin (merosin M-chain or laminin-α_2) deficient muscle status .

Based on neuroradiological and immunohistochemical investigations, the patients with 'pure' CMD can be subdivided into two subtypes: (1) CMD without white matter changes and normal merosin (merosin M-chain or laminin-α_2) expression and (2) CMD with white matter changes and a deficient merosin status.[39]

CMD type II. Fukuyama type of CMD

In the light of the recent classification for CMD,[1,2] we reviewed the data on our eight patients with CMD and impaired intellectual development, who were classified in 1989[3] and 1993[6] as having the Fukuyama type of CMD (F-CMD).[41-45] Two patients were described by Krijgsman et al in 1980.[46] The pathological findings in the central nervous system (CNS) were identical to those in the Japanese cases of F-CMD.[41-45] Four other patients were classified as F-CMD because of their clinical features, i.e. histologically proven congenital muscular dystrophy, mental retardation, neurological defects, EEG and neuroradiological (CT/MRI) abnormalities and lack of structural eye abnormalities.

In our study, normal merosin expression was found in 3 F-CMD patients. These merosin findings contrast with the findings in 17 Japanese F-CMD patients.[47] In these patients, there was on average a 74% reduction. As the gene for Japanese F-CMD has been localized on chromosome 9[48] and the merosin gene on chromosome 6, the low levels of merosin in Japanese F-CMD seem secondary to another as yet unknown defect. Thus, our results show phenotypic heterogeneity of merosin expression in F-CMD. The normal merosin results in our F-CMD patients are in agreement with a merosin gene that is functioning normally. Our findings indicate that different primary defects may be involved in F-CMD. However, according to Dobyns et al[49] another explanation for the merosin discrepancy might be that most non-Japanese F-CMD patients should be diagnosed as having muscle-eye-brain disease (Finnish type of muscle-eye-brain disease, F-MEB-D, or Walker-Warburg syndrome, WWS) because of their more severe anomalies. Gene localization studies in non-Japanese F-CMD families are needed to clarify this.

In two female siblings who did not have eye abnormalities, we found severe degenerative features in the CNS and a neuromuscular disorder with histopathological features of CMD [50] Neuropathological examination of the brain of one sibling who died at the age of 30 months, revealed subtotal loss of neurons in the cerebral and cerebellar cortex and in the ventral pons, and secondary loss of myelin in the cerebral and cerebellar subcortical white matter These neuropathological findings are more severe than those described until now in F-CMD [41 45] Moreover, neurophysiological and neuromorphological studies on the peripheral nervous system of our patients revealed a lack of large diameter fibres, a finding not described in F-CMD [41 45] Also the clinical picture was qualitatively and quantitatively more severe than any described in the classical Fukuyama type of CMD We could not prove that our cases had F-CMD, because we could not examine dystrophin-associated proteins (DAPs), especially 43 DAG which are known to be abnormally low in F-CMD patients,[51] or molecular genetic studies, especially on chromosome 9 [48] For further differentiation within the F-CMD group, molecular genetic studies are necessary

CMD type III, or the Finnish type of muscle-eye-brain disease (F-MEB-D) and CMD type IV, or the Walker-Warburg syndrome (WWS)

The Finnish type of muscle-eye-brain disease (F-MEB-D) and the Walker-Warburg syndrome (WWS) represent rare autosomal recessive disorders in which muscles, the eyes and brain are affected Both have congenital muscular dystrophy, cobblestone (previously type II) lissencephaly and white matter changes The latter are diffuse and severe in WWS, but patchy in F-MEB-D Most WWS patients have hydrocephalus and anterior chamber malformations [1 2 49 52 55] High myopia can be present in both In F-MEB-D retinal abnormalities associated with reduced or absent responses to electroretinography (ERG) and very high amplitude responses to visual evoked potentials (VEPs) are present by 2 years of age [1 2 49 52 55] Microphthalmia, typical retinal dysplasia and corneal opacities, present in WWS, have not been observed in F-MEB-D [49]

F-MEB-D and WWS are usually classified separately because the clinical features and the neuropathological findings in WWS are more severe than in F-MEB-D[1 2 49 55 57] and the eye abnormalities are somewhat different in both diseases Progressive ERG and VEP changes have been reported in F-MEB-D but not in WWS The differences in central nervous system abnormalities all appear to be quantitative, especially the severity of the gyral malformation and

the white matter changes. In F-MEB-D patients, the gyral malformation usually consists of pachygyria over the frontal region and polymicrogyria posteriorly. The white matter changes are present but patchy in about half of the F-MEB-D patients. Most WWS patients have more severe gyral changes, consisting of agyria or mixed agyria-pachygyria with some areas of polymicrogyria. All have severe and diffuse white matter changes. Cerebellar hypoplasia might be somewhat more severe in WWS. Also the occipital cephaloceles which occur in about 30% of patients with WWS, have been reported very rarely in F-MEB-D. All these elements constitute a qualitative difference, but Dobyns is of the opinion that there is also a quantitative difference. Thus, severity forms the main difference between F-MEB-D and WWS, but this is not a reliable way to differentiate between these two genetic syndromes.[1,2,49,55]

Our six patients with congenital muscular dystrophy and involvement of the central nervous system and eyes, who we classified as 'muscle-eye-brain disease' (MEB-D) in 1992,[5] correspond with what now is called the (more severe) WWS and not with F-MEB-D, which is milder. According to the literature,[49,55,58-61] progression of the disease is rapid in most WWS patients; this was also the case in five out of our six patients.[5] In contrast, the patients in Santavuori's study showed slow progression, typical for F-MEB-D.[57] Considering the clinical and neuropathological expression and progression patterns, our study supports the notion that the disease exists in different levels of severity, ranging from a milder type in the F-MEB-D group to a more severe type in the WWS group.[49,55]

Creatine kinase (CK) values

In agreement with literature reports,[1,2,8-24] most of our patients with 'pure' CMD without white matter hypodensity, had normal or moderately increased serum CK values (up to 4 times the upper limit of the reference range). The patients in the study by Topaloğlu et al[37] with 'pure' CMD and white matter hypodensity, had significantly higher values of CK (ranging from normal up to 17 times the upper limit of the reference range) in comparison with their group of 'pure' CMD patients without white matter hypodensity (ranging from normal up to 4 times the upper limit of the reference range). Our patients with 'pure' CMD and white matter hypodensity also had normal or moderately increased CK values (up to 13 times the upper limit of the reference range).[4] In the literature, the CK values in F-CMD were significantly elevated in all cases (10 to 50 times the upper limit of the reference range).[41,42] Our F-CMD patients had normal or 6 to

7 times the normal CK values.[3,6] In the literature, CK values in F-MEB-D were significantly elevated in all cases (3 to 20 times the upper limit of the reference range),[36,57] as well as in WWS (variable values, ranging from 3 to 60 times the upper limit of the reference range)[55] and could vary greatly between patients or over time in an individual patient. Our WWS patients also had elevated CK values (10 to 16 times the upper limit of the reference range).[5] Considering the CK values in CMD, it is our impression that the more organs involved, the higher the CK value.

Muscle biopsy

The histomorphological findings in the muscle biopsy specimens from our 'pure' CMD patients with and without low density areas in the white matter and in the F-CMD and WWS patients, did not differ essentially.[6] It was only possible to observe increasing fat cell infiltration with increasing age in our 'pure' CMD series without white matter hypodensity. Immunohistochemistry with dystrophin, vimentin and desmin antibodies showed a normal expression pattern.[6] Merosin (merosin M-chain or laminin-α_2) expression was normal in eleven patients (six had 'pure' CMD without white matter hypodensity, three were considered to have F-CMD and two had WWS), was severely decreased in two cases with 'pure' CMD with white matter hypodensity and showed variable expression in one of two exceptional F-CMD-like cases.[50,62] Tomé et al[63] and Mercuri et al[39] were the first authors to report the absence of merosin in some cases of 'pure' CMD, whereas Hayashi et al[47] reported near-normal values by quantitative immunofluorescence in 'pure' CMD. All 10 merosin-positive cases in the study by Mercuri et al[39] had normal MRI of the CNS, whereas all the merosin-negative cases had moderate to severe diffuse white matter changes on MRI. The study by Mercuri et al[39] was the first to make a clear distinction between patients with 'pure' CMD without white matter hypodensity but with normal merosin expression and 'pure' CMD with white matter hypodensity plus a deficient merosin status. In our material on 'pure' CMD,[62] only two cases had merosin deficiency; they also had white matter hypodensity, which is in agreement with the findings in the study by Mercuri et al.[39]

In the study by Hayashi et al,[47] quantitative immunofluorescence showed that the merosin content in F-CMD was reduced to a mean of 26%. However, merosin (merosin M-chain or laminin-α_2) expression was normal in intrafusal fibres, which suggests a secondary change.[40] In contrast, in our study, normal merosin (merosin M-chain or laminin-α_2) expression was found in the three

patients considered to have F-CMD [62] As the gene for Japanese F-CMD has been localized on chromosome 9q31-33[48] and the merosin gene on chromosome 6q2,[2 64] these low levels of merosin in Japanese F-CMD should be considered as secondary to another as yet unknown defect Thus, the differences in merosin expression between Japanese and non-Japanese F-CMD suggest genetic heterogeneity However, another explanation is that some of our F-CMD patients may have F-MEB-D or a mild form of WWS Dobyns et al[49] believe that most non-Japanese patients diagnosed as having F-CMD have more severe anomalies typical of F-MEB-D or WWS, or less severe anomalies consistent with CMD with mental retardation In a recent study, consistently preserved merosin (merosin M-chain or laminin-α_2) expression was found in five patients with WWS, as detected by immunofluorescence in skeletal muscle [40] This is in accordance with our findings The authors claimed that merosin can be used as an immunocytochemical marker to distinguish WWS from F-CMD

Electron microscopy examination of the muscle biopsy of the father in our family with possible dominant 'pure' CMD[65] showed mitochondria with crystalline inclusions in a large group of fibres and proliferation of concentric cristae [65] Both patients in the study by Kalyanaraman et al[66] had mitochondrial abnormalities in their muscle tissue (biopsy), i e an increased number of mitochondria of abnormal size and shape with distorted cristae Although this may be an unspecific or age-related finding, these observations indicate that in the future, more attention should be paid to the exploration of mitochondria in CMD

Genetic aspects

In general, it is agreed that CMD, F-CMD, F-MEB-D and WWS have an autosomal recessive pattern of inheritance [1 2 8 24 41 45 49 52 57] Recent studies have indicated that in 'pure' congenital muscular dystrophy, a proportion of cases are merosin-negative The locus for the merosin gene is on chromosome 6q2 [2 64] The gene responsible for typical F-CMD has recently been mapped to chromosome 9q31-33 [48] Linkage studies on ten Finnish families with F-MEB-D excluded the F-MEB-D gene from this region [67] Toda et al[68] claimed that F-CMD and WWS are genetically identical Their findings were based on microsatellite marker analysis of two siblings, one presumed to be suffering from F-CMD, one from WWS Both cases showed the same alleles for the flanking markers of the F-CMD locus on 9q31-33 No statistical analysis or lod score calculation of these results were provided and no mutation of the F-CMD gene was demonstrated [40]

Many explanations can account for this observation. However, these findings contrast with the different merosin (merosin M-chain or laminin-α_2) expression in the skeletal muscle of F-CMD versus WWS.[40]

Kalyanaraman et al[66] are the only authors to have reported on a family with CMD and possible CNS involvement (i.e. seizures in the mother and poor reading ability with an abnormal EEG (spike and wave complexes) in the son), which suggests an autosomal dominant pattern of inheritance. We examined a family (father and daughter) with 'pure' CMD in which inheritance was possibly autosomal dominant.[65] Theoretically, the mother in our family could be a carrier (heterozygote) of an autosomal recessive type of CMD.

In conclusion, for definite delineation of the nosological entities of CMD, further molecular genetic research is necessary.

Particular forms

In our series, there were two particular forms of congenital muscular dystrophy which we could not classify. One child had a combination of congenital muscular dystrophy, hypoplasia of the lateral abdominal wall musculature (especially of the oblique abdominal musculature) and hypoplasia of the external genitalia. This case illustrates a rare combination of a muscular dystrophic process and a muscular developmental defect.[69] The other patient, a boy, had unilateral isolated dystrophic diaphragmatic musculature.[70] In the literature, only Bergen et al[71] described a neonate with isolated congenital dystrophy of the diaphragm.

Severe degenerative features of the central nervous system were reported in two female siblings with CMD. Brain pathology differed essentially from that described previously in conjunction with CMD, i.e. subtotal loss of neurons in the cerebral and cerebellar cortex and in the ventral pons, and secondary loss of myelin in the cerebral and cerebellar subcortical white matter. Sural nerve pathology showed a lack of large diameter fibres, a finding not described in F-CMD. There was a marked variability of merosin (merosin M-chain or laminin-α_2) expression in the skeletal muscle fibres in one of these two patients, as is also found in Japanese F-CMD. Within the spectrum of F-CMD these cases may illustrate a more severe type or they may represent a distinct entity.

In our latest report, we described three siblings (one sister and two brothers) with an adult-onset of predominantly distal muscle weakness.[72] In the female index patient, this was associated with epilepsy and a progressive spastic ataxic gait, while the two other siblings had no appreciable clinical nervous system involvement. Additional investigations revealed muscular dystrophy and leuko-

encephalopathy in all three siblings. After the publication of this article, merosin expression was found to be normal in the one patient we examined. Only by excluding abnormalities in the merosin (merosin M-chain or laminin-α_2) (6q2) gene, can we conclude that this is a different type of muscular dystrophy.

Conclusion

This study indicates how fast research into CMD has developed in recent years. Molecular genetic research ('genetic mapping') has contributed greatly to research into dystrophinopathies. Associating clearly delineated clinical signs and symptoms with morphological and genetic data will help to establish a better defined classification for syndromes with congenital muscular dystrophy. Our study supports the recent CMD classification (see Table), but suggests that more subtypes may be identified in the future.

Table. The 1995 CMD classification according to the literature and two ENMC sponsored workshops[1,2]

CMD I	Classical or 'pure' CMD without severe impairment of intellectual development: **A.** Without white matter hypodensity and with normal merosin (merosin M-chain or laminin-α_2) expression (McKusick, no. 23667), **B.** With white matter hypodensity and with deficient merosin (merosin M-chain or laminin-α_2) status.
CMD II	CMD with mental impairment due to structural brain abnormality: the Fukuyama type (McKusick, no. 253800).
CMD III	CMD with eye and brain abnormalities: the Finnish type (McKusick, no. 253280).
CMD IV	CMD with eye and brain abnormalities: the Walker-Warburg syndrome (McKusick, no. 236670).

References

1. Dubowitz V. Workshop report: 22nd ENMC sponsored workshop on congenital muscular dystrophy held in Baarn, The Netherlands, 14-16 May 1993. Neuromusc Disord 1994;4:75-81.
2. Dubowitz V, Fardeau M. Workshop report: proceedings of the 27th ENMC sponsored workshop on congenital muscular dystrophy. 22-24 April 1994, The Netherlands. Neuromusc Disord, 1995;5:253-258.
3. Leyten QH, Gabreëls FJM, Renier WO, Ter Laak HJ, Sengers RCA, Mullaart RA. Congenital muscular dystrophy. J Pediatr 1989;115:214-221.
4. Leyten QH, Gabreëls FJM, Renier WO, Van Engelen BGM, Ter Laak HJ, Sengers RCA, Thijssen HOM. White matter abnormalities in congenital muscular dystrophy. J Neurol Sci 1995;129:162-169.
5. Leyten QH, Gabreëls FJM, Renier WO, Renkawek K, Ter Laak HJ, Mullaart RA. Congenital muscular dystrophy with eye and brain malformations in six Dutch patients. Neuropediatrics 1992;23:316-320.
6. Leyten QH, Ter Laak HJ, Gabreëls FJM, Renier WO, Renkawek K, Sengers RCA. Congenital muscular dystrophy: a study on the variability of morphological changes and dystrophin distribution in muscle biopsies. Acta Neuropathol (Berl) 1993;86:386-392.
7. Leyten QH, Renkawek K, Renier WO, Gabreëls FJM, Mooy CM, Ter Laak HJ, Mullaart RA. Neuropathological findings in muscle-eye-brain disease (MEB-D): neuropathological delineation of MEB-D from congenital muscular dystrophy of the Fukuyama type. Acta Neuropathol (Berl) 1991;83:55-60.
8. Rotthauwe HW, Kowalewski S, Mumenthaler M. Kongenitale Muskeldystrophie. Z Kinderheilkd 1969;106:131-162.
9. Donner M, Rapola J, Somer H. Congenital muscular dystrophy: a clinicopathological and follow-up study of 15 patients. Neuropädiatrie 1975;6:239-258.
10. Lazaro RP, Fenichel GM, Kilroy AW. Congenital muscular dystrophy: case and reappraisal. Muscle Nerve 1979;2:349-355.
11. McMenamin JB, Becker LE, Murphy EG. Congenital muscular dystrophy: a clinicopathologic report of 24 cases. J Pediatr 1982;100:692-697.
12. Banker BQ. Congenital muscular dystrophy. In: Engel AG, Banker BQ, eds. Myology, vol 2. New York: McGraw-Hill, 1986, pp 1367-1382.
13. Serratrice G, Cros D, Pellissier J-F, Gastaut J-L, Pouget J. Dystrophie musculaire congénitale. Rev Neurol (Paris) 1980;136:445-472.

14. Bethlem J. Congenital muscular dystrophy. In: Bethlem J, ed. Myopathies. Amsterdam, New York: North Holland Publ. Co, 1977, pp 91-92.
15. Brooke MH. Congenital muscular dystrophy. In: Brooke MH, ed. A clinician's view of neuromuscular diseases, 2nd ed. Baltimore: Williams & Wilkins, 1986, pp 345-345.
16. Brooke MH. Congenital muscular dystrophy. In: Bradley WG, Daroff RB, Fenichel GM, Marsden CD, eds. Neurology in clinical practice, vol II. Boston: Butterworth-Heinemann, 1991, pp 1882-1884.
17. Dubowitz V, Brooke MH. Congenital muscular dystrophy. In: Dubowitz V, Brooke MH, eds. Muscle biopsy: a practical approach, 2nd ed. London: Saunders, 1985, pp 370-380.
18. Dubowitz V. Congenital muscular dystrophy. In Dubowitz V, ed. Muscle diseases in childhood, 2nd ed. Chicago: Year Book Med Publ, 1989, pp 52-65.
19. Fardeau M. Congenital muscular dystrophy. In: Mastaglia FL, Walton J, eds. Skeletal muscle pathology. Edinburgh: Churchill-Livingstone, 1982, pp 184-186.
20. Fenichel GM. Congenital muscular dystrophies. Neurol Clin 1988:6:519-528.
21. Lenard H-G. Congenital muscular dystrophies: problems of classification. Acta Paediatr Jpn (Tokyo) 1991;33:256-260.
22. Walton JN, Gardner-Medwin D. Congenital muscular dystrophy, hypotonic sclerotic muscular dystrophy (Ullrich), Fukuyama muscular dystrophy. In: Walton N, ed. Disorders of voluntary muscle, 5th ed. Edinburgh: Churchill-Livingstone, 1988, pp 548-551.
23. Oliveira AS, Gabbai AA, Kiyomoto BH, Ferreira-Neto A, Schmidt B, Lima JG. Congenital muscular dystrophy: clinical study of 17 patients. Arq Neuropsiquiatr 1991:49:265-271.
24. Parano E, Fiumara A, Falsperla R, Vita G, Trifiletti RR. Congenital muscular dystrophy: correlation of muscle biopsy and clinical features. Pediatr Neurol 1994;10:233-236.
25. Bernier J-P, Brooke MH, Naidich TP, Carroll JE. Myoencephalopathy: cerebral hypomyelination revealed by CT scan of the head in a muscle disease. Trans Am Neurol Assoc 1979;104:244-246.
26. Nogen AG. Congenital muscle disease and abnormal findings on computerized tomography. Dev Med Child Neurol 1980;22:658-663.

27. Egger J, Kendall BE, Efdohazi M, Lake BD, Wilson J, Brett EM. Involvement of the central nervous system in congenital muscular dystrophy. Dev Med Child Neurol 1983;25:32-42.
28. Echenne B, Arthuis M, Billard C, Campos-Castello J, Castel Y, Dulac O, Fontan D, Gauthier A, Kulakowski S, De Meuron G, Moore JR, Nieto-Barrera M, Pages M, Parain D, Pavone L, Ponsot G. Congenital muscular dystrophy and cerebral CT scan anomalies: results of a collaborative study of the Société de Neurologie Infantile. J Neurol Sci 1986;75:7-22.
29. Martinelli P, Gabellini AS, Ciucci G, Govoni E, Vitali S, Gulli MR. Congenital muscular dystrophy with central nervous system involvement: case report. Eur Neurol 1987;26:17-22.
30. Yoshioka M, Kuroki S, Mizue H. Congenital muscular dystrophy of non-Fukuyama type with characteristic CT images. Brain Dev 1987;9:316-318.
31. Castro-Gago M, Peña-Guitián J. Congenital muscular dystrophy of a non-Fukuyama type with characteristic CT images (letter to the editor). Brain Dev 1988;10:60.
32. Streib EW, Lucking CH. Congenital muscular dystrophy with leuko-encephalopathy. Eur Neurol 1989;29:211-215.
33. Tanaka J, Mimaki T, Okada S, Fujimura H. Changes in cerebral white matter in a case of congenital muscular dystrophy (non-Fukuyama type). Neuropediatrics 1990;21:183-186.
34. Cook JD, Gascon GG, Haider A, Coates R, Stigsby B, Ozand PT, Banna M. Congenital muscular dystrophy with abnormal radiographic myelin pattern. J Child Neurol 1992;7(Suppl):51-63.
35. Kacinski M, Bando B, Kroczka K, et al. A case of congenital muscular dystrophy with changes in the white matter of the brain. Neurol Neurochir Pol 1992;26:383-387.
36. Pihko H, Louhimo T, Valanne L, Donner M. CNS in congenital muscular dystrophy without mental retardation. Neuropediatrics 1992;23:116-122.
37. Topaloğlu H, Kale G, Yalnizoğlu D, Taşdemir AH, Karaduman A, Topçu M, Kotiloğlu E. Analysis of "pure" congenital muscular dystrophies in thirty-eight cases. How different is the classical type 1 from the occidental type cerebromuscular dystrophy? Neuropediatrics 1994;25:94-100.
38. Malik S, Cruse RP, Chou SM, Chafel T. Non-Fukuyama congenital muscular dystrophy with central nervous system involvement: clinical, radiographic and pathological correlates (abstract). Ann Neurol 1990;28:430.
39. Mercuri E, Muntoni F, Berardinelli A, Pennock J, Sewry C, Philpot J, Dubowitz V. Somatosensory and visual evoked potentials in congenital

muscular dystrophy: correlation with MRI changes and muscle merosin status. Neuropediatrics 1995;26:3-7.
40. Voit T, Sewry CA, Meyer K, Hermann R, Straub V, Muntoni F, Kahn T, Unsöld R, Helliwell TR, Appleton R, Lenard HG. Preserved merosin M-chain (or laminin-α_2) expression in skeletal muscle distinguishes Walker-Warburg syndrome from Fukuyama muscular dystrophy and merosin-deficient congenital muscular dystrophy. Neuropediatrics 1995;26:148-155.
41. Fukuyama Y, Kawazura M, Haruna H. A peculiar form of congenital progressive muscular dystrophy: report of fifteen cases. Pediatr Univ Tokyo (Tokyo) 1960;4:5-8.
42. Fukuyama Y, Osawa M, Suzuki H. Congenital progressive muscular dystrophy of the Fukuyama type: clinical, genetic and pathological considerations. Brain Dev 1981;3:1-29.
43. Fukuyama Y, Osawa M. Congenital muscular dystrophy: clinico-nosological aspects. In: Ebashi S, ed. Muscular dystrophy. Proceedings of the international symposium of muscular dystrophy, held November 25-27, 1980, in Tokyo. Tokyo: University of Tokyo Press, 1982, pp 399-424.
44. Osawa M, Arai Y, Ikenaka H, Murasugi H, Sugahara N, Sumida S, Okada N, Shishikura K, Suzuki H, Hirayama Y, Hirasawa K, Fukuyama Y, Tsutsumi A, Ito K, Uchida Y. Fukuyama type congenital progressive muscular dystrophy. Acta Paediatr Jpn (Tokyo) 1991;33:261-269.
45. Kamoshita S, Konishi Y, Segawa M, Fukuyama Y. Congenital muscular dystrophy as a disease of the central nervous system. Arch Neurol 1976; 33:513-516.
46. Krijgsman JB, Barth PG, Stam FG, Sloof JL, Jaspar HHJ. Congenital muscular dystrophy and cerebral dysgenesis in a Dutch family. Neuropädiatrie 1980;11:108-120.
47. Hayashi YK, Engvall E, Arikawa-Hirasawa E, Goto K, Koga R, Nonaka I, Sugita H, Arahata K. Abnormal localization of laminin subunits in muscular dystrophies. J Neurol Sci 1993;119:53-64.
48. Toda T, Segawa M, Nomura Y, Nonaka I, Masuda K, Ishihara T, Suzuki M, Tomita I, Origuchi Y, Ohno K, Misugi N, Sasaki Y, Takada K, Kawai M, Otani K, Murakami T, Saito K, Fukuyama Y, Shimizu T, Kanazawa I, Nakamura Y. Localization of a gene for Fukuyama type congenital muscular dystrophy to chromosome 9q31-33. Nature Genetics 1993;5:283-286.
49. Dobyns WB, Truwit CL. Lissencephaly and other malformations of cortical development: 1995 update. Neuropediatrics 1995;26:132-147.

50. Leyten QH, Barth PG, Gabreëls FJM, Renkawek K, Renier WO, Gabreëls-Festen AAWM, Ter Laak HJ, Smits MG. Congenital muscular dystrophy and severe central nervous system atrophy in two siblings. Acta Neuropathol 1995, in press.
51. Matsumara K, Nonaka I, Campbell KP. Abnormal expression of dystrophin-associated proteins in Fukuyama-type congenital muscular dystrophy. Lancet 1993;341:521-522.
52. Walker AE. Lissencephaly. Arch Neurol Psychiat 1942;48:13-29.
53. Warburg M. Heterogeneity of congenital retinal non-attachment, falciform folds and retinal dysplasia: a guide to genetic counselling. Hum Hered 1976;26:137-148.
54. Warburg M. Hydrocephaly, congenital retinal nonattachment, and congenital falciform fold. Am J Ophthalmol 1978;85:88-94.
55. Dobyns WB, Pagon RA, Armstrong D, Curry CJR, Greenberg F, Grix A, Holmes LB, Laxova R, Michels VV, Robinow M, Zimmerman RL. Diagnostic criteria for Walker-Warburg syndrome. Am J Med Genet 1989;32: 195-210.
56. Raitta C, Lamminen M, Santavuori P, Leisti J. Ophthalmological findings in a new syndrome with muscle, eye and brain involvement. Acta Ophthalmol (Copenh) 1978;56:465-472.
57. Santavuori P, Somer H, Sainio K, Rapola J, Kruus S, Nikitin T, Ketonen L, Leisti J. Muscle-eye-brain disease (MEB). Brain Dev 1989;11:147-153.
58. Bordarier C, Aicardi J, Goutieres F. Congenital hydrocephalus and eye abnormalities with severe developmental brain defects: Warburg's syndrome. Ann Neurol 1984;16:60-65.
59. Attia MFD, Burn J, McCarthy JH, Purohit DP, Milligan DWA. Warburg (HARD ± E) syndrome without retinal dysplasia: case report and review. Br J Ophthalmol 1986;70:742-747.
60. Heggie P, Grossniklaus HE, Roessmann U, Chou SM, Cruse RP. Cerebro-ocular dysplasia-muscular dystrophy syndrome: report of two cases. Arch Ophthalmol 1987;105:520-524.
61. Towfighi J, Sassani JW, Suzuki K, Ladda RL. Cerebro-ocular dysplasia-muscular dystrophy (COD-MD) syndrome. Acta Neuropathol (Berl) 1984; 65:110-123.
62. Ter Laak HJ, Leyten QH, Gabreëls FJM, Kuppen H, Renier WO, Sengers RCA. Abnormal merosin (or laminin-α_2) expression in congenital muscular dystrophies: an immunohistochemical study. 1995, submitted for publication.

63. Tomé FMS, Evangelista T, Leclerc A, Sunada Y, Manole E, Estournet B, Barois A, Campbell KP, Fardeau M. Congenital muscular dystrophy with merosin deficiency. C R Acad Sci Paris, Sciences de la Vie (Life Sciences), 1994;317:351-357.
64. Hillaire D, Leclerc A, Fauré S, Topaloglu H, Chiannilkulchaï N, Guicheney P, Grinas L, Legos P, Philpot J, Evangelista T, Routon M-C, Mayer M, Pelissier J-F, Estournet B, Barois A, Hentati F, Feingold N, Beckmann JS, Dubowitz V, Tomé FMS, Fardeau M. Localization of merosin-negative congenital muscular dystrophy to chromosome 6q2 by homozygosity mapping. Hum Mol Genet 1994;3:1657-1661.
65. Leyten QH, Gabreëls FJM, Joosten EMG, Renier WO, Ter Laak HJ, Ter Haar BGA, Stadhouders AM. An autosomal dominant type of congenital muscular dystrophy. Brain Dev 1986;8:533-537.
66. Kalyanaraman K, Kalyanaraman UP, Lee RH. Autosomal dominant congenital muscular dystrophy (CMD) with possible central nervous system (CNS) involvement (abstract). J Neuropathol Exp Neurol 1980;39:366.
67. Ranta S, Pihko H, Santavuori P, Tahvanainen E, De la Chapelle A. Muscle-eye-brain disease and Fukuyama type congenital muscular dystrophy are not allelic. Neuromusc Disord 1995, in press.
68. Toda T, Yoshioka M, Nakahori Y, Kanazawa I, Nakamura Y, Nakagome Y. Genetic identity of Fukuyama-type congenital muscular dystrophy and Walker-Warburg syndrome. Ann Neurol 1995;37:99-101.
69. Leyten QH, Gabreëls FJM, Renier WO, Brunner HG, Ter Laak HJ, Mullaart RA. Association of congenital muscular dystrophy, hypoplasia of the lateral abdominal wall musculature and hypoplasia of the external genitalia. Neuropediatrics 1995, accepted for publication.
70. Leyten QH, Renier WO, Gabreëls FJM, Ter Laak HJ, Hinkofer LHA. Dystrophic myopathy of the diaphragm in a neonate with severe respiratory failure during infectious episodes. Neuromusc Disord 1993;3:51-55.
71. Bergen BJ, Sangalang VE, Aterman K, Isolated myopathic involvement of the diaphragmatic musculature in a neonate. Ann Neurol 1977;1:403-407.
72. Van Engelen BGM, Leyten QH, Bernsen PLJA, Gabreëls FJM, Barkhof F, Joosten EMG, Hamel BCJ, Ter Laak HJ, Ruijs NBM, Cruysberg JRM, Valk J. Familial adult-onset muscular dystrophy with leukoencephalopathy. Ann Neurol 1992;32:577-580.

SUMMARY

This study on congenital muscular dystrophy (CMD) focusses on the clinical and morphological aspects of the disorder.

In Chapter II, the literature is reviewed within the framework of recent CMD classification. This study aims to evaluate and delineate the clinical and morphological characteristics of the different types of CMD by comparing our findings to data reported in the literature.

In the past, CMD was often used as a 'catch-all' diagnosis owing to its imprecise delineation and the confusing overlap of conditions. Furthermore, there were several forms of CMD, with a wide range of clinical findings and variable prognoses, which shared similar histological muscle changes. The clinical expression of CMD was determined by the presence or absence of anomalies of the brain and eyes. The prognosis was variable and depended on the severity of the disease.

The myopathological manifestations of congenital muscular dystrophy were clear, but there were no differentiating histological and electron microscopic aspects. The changes were variable and included features like variation in fibre size, the presence of necrotic and basophilic fibres, an increased number of fibres with internal nuclei and the substitution of muscle tissue by endomysial fat and connective tissue.

Signs and symptoms, clinical course, laboratory, neurophysiological, radiological, morphological and genetic characteristics are outlined for each subcategory. Histological characteristics of muscle biopsy specimens and neuropathological findings in the central nervous system (CNS) and the eyes are described for each subcategory. The present-day discussion about whether the Fukuyama type of CMD (F-CMD), the Finnish type of muscle-eye-brain disease (F-MEB-D) and the Walker-Warburg syndrome (WWS) should be considered to represent a spectrum of one disease, is continued at the end of this Chapter. Although there is consensus that the same or a similar basic pathological process is involved, there are also several consistent and mainly quantitative differences between these entities. Especially merosin (merosin M-chain or laminin-α_2) has accentuated the discussion.

Chapter III section 1.1 reviews eighteen patients with congenital muscular dystrophy. Six of them also have involvement of the central nervous system that corresponded with the Fukuyama type of congenital muscular dystrophy. In four patients, both the CNS and the eyes are involved, so the diagnosis of 'muscle, eye and brain disease' has been made. With reference to the 1995 proposal for the classification of CMD, our four patients described as having muscle-eye-

brain disease in 1989, should now be classified under the Walker-Warburg syndrome.

Chapter III section 1.2 presents the clinical signs, symptoms and central nervous system characteristics (intelligence, cerebrospinal fluid, especially myelin basic protein, EEG and neuroradiological findings (CT/MRI)) of 17 patients with classic or 'pure' congenital muscular dystrophy. In three patients, neuroradiological examinations (CT/MRI) indicate hypodense white matter areas. Two out of the three have epilepsy. In our opinion, the classic or 'pure' form can be subdivided into two subtypes, i.e. those with and those without white matter hypodensities. Based on our study and the literature data, a mild form of epilepsy or an epileptic predisposition on the EEG can be part of the subtype with white matter hypodensities.

In Chapter III section 1.3, six patients with muscle, eye and brain disease (WWS according to the 1995 classification) are reported on. Progression of the disease has been rapid in five out of the six patients. Our data are in agreement with those recently published in the literature.

Chapter III section 2.1 describes the morphological variability in muscle specimens obtained from thirty patients with congenital muscular dystrophy (fifteen with 'pure' CMD, two with 'pure' CMD with hypodense white matter on CT/MRI, six with F-CMD, two exceptional cases with Fukuyama-like CMD and five with WWS). No morphological hallmarks were found to differentiate between these subgroups. Fat cell infiltration was only found to be increased with increasing age in 'pure' CMD. Immunohistochemistry with dystrophin, vimentin and desmin antibodies in fourteen patients showed a normal expression pattern. Immunohistochemistry with merosin (Chapter III section 2.3) in thirteen patients revealed a normal expression pattern in eleven of them (6 'pure' CMD, 3 F-CMD, 2 WWS), but there was little or no expression in two patients with 'pure' CMD and white matter hypodensity.

In Chapter III section 2.2, neuropathological findings are described in two sisters with muscle, eye and brain disease (WWS type). Pathological examination demonstrated muscular dystrophy, hydrocephalus, cobblestone (previously called type II) lissencephaly, dysplasia of the cerebellum, agenesis of the vermis, Dandy-Walker cyst and defective eye development of fetal origin.

In Chapter IV, particular forms of CMD are presented which cannot be classified within the new classification system. In Chapter IV section 1.1, a family is described with a (probable?) autosomal dominant type of congenital muscular dystrophy. This report concerns a father and daughter suffering from

CMD without CNS involvement. The histological findings, especially some mitochondrial abnormalities in the muscle biopsy of the father, are remarkable.

In Chapter IV section 1.2, severe degenerative features of the central nervous system are reported in two female siblings, associated with a neuromuscular disorder with histological features of CMD. Brain pathology differed essentially from that described previously in conjunction with CMD, i.e. subtotal loss of neurons in the cerebral cortex, cerebellar cortex and in the ventral pons and secondary loss of myelin in the cerebral and cerebellar subcortical white matter. These pathological changes are considered to reflect very early multisystem degeneration of the CNS. Sural nerve pathology manifested a lack of large diameter fibres, a finding not described in F-CMD. Within the spectrum of F-CMD, these cases may illustrate an extremely severe type.

In Chapter IV section 1.3, a Dutch girl is presented with congenital muscular dystrophy associated with hypoplasia of the lateral abdominal wall musculature and hypoplasia of the external genitalia.

In Chapter IV section 2.1, a Dutch boy is presented with an isolated dystrophic diaphragmatic musculature, who showed slight postnatal asphyxia and later on, episodes of respiratory insufficiency when viral infections occurred.

In Chapter IV section 3.1, three siblings are reported with adult-onset, predominantly distal muscle weakness. In the female index patient, epilepsy and a spastic ataxic gait are present, while the two other siblings show no appreciable clinical central nervous system involvement. All three siblings have muscular dystrophy and leukoencephalopathy.

In Chapter V, General Discussion, our results are reconsidered in the light of the most recent classification of CMD from 1995.

SAMENVATTING

Deze studie over congenitale spierdystrofie (CMD) richt zich vooral op de klinische en morfologische aspecten van de aandoening.

In Hoofdstuk II wordt een overzicht gegeven van de literatuur in het kader van de recente CMD classificatie. Met deze studie hebben wij getracht te komen tot een evaluatie en beschrijving van de klinische en morfologische kenmerken van de verschillende typen van CMD middels vergelijking van onze resultaten met de literatuurgegevens.

In het verleden werd CMD vaak gebruikt als een 'alles omvattende' diagnose, aangezien de verschijningsvorm niet nauwkeurig was en de 'overlap' van de criteria verwarrend. Bovendien bestonden er verscheidene vormen van CMD met en breed scala aan klinische bevindingen en wisselende prognose, welke gelijksoortige histologische spierveranderingen deelden. De klinische expressie van CMD werd bepaald door de aan- of afwezigheid van afwijkingen aan hersenen en ogen. De prognose was wisselend en afhankelijk van de ernst van de ziekte.

De aanwezigheid van spierafwijkingen bij CMD was duidelijk, maar onderscheid in histochemische en electronenmicroscopische kenmerken ontbrak. De afwijkingen waren variabel en omvatten kenmerken als variatie in vezelgrootte, de aanwezigheid van necrotische en basofiele vezels, een toegenomen aantal vezels met interne nuclei en vervanging van spierweefsel door endomysiaal vet- en bindweefsel.

Van elke afzonderlijke categorie worden de klachten en verschijnselen, klinisch beloop, laboratorium, neurofysiologische, radiologische, morfologische en genetische kenmerken uiteengezet. De histologische kenmerken van de spierbiopten en de neuropathologische bevindingen in het centrale zenuwstelsel en de ogen worden voor elke categorie achtereenvolgens beschreven. De huidige discussie of Fukuyama type CMD (F-CMD), het Finse type van 'muscle-eye-brain disease' (F-MEB-D) en het Walker-Warburg syndroom (WWS) als een spectrum van één ziekte dienen te worden beschouwd, wordt besproken aan het eind van dit hoofdstuk. Ofschoon er consensus bestaat over het feit dat er sprake is van eenzelfde of gelijksoortig fundamenteel pathologisch proces, bestaan er ook verscheidene consistente, voornamelijk kwantitatieve verschillen tussen deze entiteiten. Vooral merosine (merosin M-chain or laminin-α_2) heeft de discussie geaccentueerd.

Hoofdstuk III paragraaf 1.1 bevat een overzichtsverslag van achttien patiënten met congenitale spierdystrofie, waarvan er zes ook een aandoening hebben van het centrale zenuwstelsel, overeenkomend met Fukuyama type CMD. Bij vier patiënten zijn zowel het centrale zenuwstelsel als de ogen aangedaan; op grond waarvan de diagnose 'muscle-eye-brain disease' is gesteld. Onder verwijzing

naar het voorstel uit 1995 met betrekking tot de classificatie van CMD dienen onze vier patiënten welke in 1989 werden beschreven als 'muscle-eye-brain disease', thans te worden geclassificeerd als Walker-Warburg syndroom.

In Hoofdstuk III paragraaf 1.2 worden de klinische verschijnselen alsook kenmerken van het centrale zenuwstelsel (intelligentie, liquor cerebrospinalis, met name het basisch myeline eiwit, electroencephalogram en neuroradiologische bevindingen) gepresenteerd van zeventien patiënten met klassieke of 'pure' CMD. Neuroradiologisch onderzoek (CT/MRI) laat bij drie patiënten hypodense gebieden in de witte stof zien. Twee van deze drie patiënten hebben epilepsie. We zijn van mening dat de klassieke of 'pure' vorm kan worden onderverdeeld in twee subtypes, namelijk één met en één zonder hypodense gebieden in de witte stof. Op grond van onze studie en de literatuurgegevens kan een lichte vorm van epilepsie of een epileptische predispositie op het EEG deel uitmaken van het subtype met hypodense gebieden in de witte stof.

In Hoofdstuk III paragraaf 1.3 worden zes patiënten beschreven met 'muscle-eye-brain disease' (WWS volgens de classificatie van 1995). Bij vijf van de zes patiënten heeft de ziekte een snel progressief beloop. Onze gegevens zijn in overeenstemming met die van de recente literatuur.

In Hoofdstuk III paragraaf 2.1 wordt de morfologische variabiliteit bestudeerd in de spierbiopten van dertig patiënten met congenitale spierdystrofie (vijftien met 'pure' CMD, twee met 'pure' CMD en hypodense gebieden in de witte stof op CT/MRI, zes met F-CMD, twee uitzonderlijke patiënten met CMD gelijkend op Fukuyama type CMD, en vijf met WWS). Er werden geen morfologische kenmerken gevonden welke deze subgroepen van elkaar kunnen onderscheiden. Alleen een met de leeftijd toenemende vetcel infiltratie werd aangetroffen bij 'pure' CMD. Immunohistochemie met dystrofine-, vimentine- en desmine- antilichamen toonde bij veertien patiënten een normaal patroon. Immunohistochemie met merosine (Hoofdstuk III paragraaf 2.3) toonde een normaal patroon bij elf van de dertien onderzochte patiënten (zes met 'pure' CMD, drie met F-CMD en twee met WWS) en was nagenoeg afwezig bij twee patiënten met 'pure' CMD en hypodense gebieden in de witte stof.

In Hoofdstuk III paragraaf 2.2 worden de neuropathologische bevindingen beschreven van twee zusters met 'muscle-eye-brain disease' (WWS type). Pathologisch onderzoek toonde spierdystrofie, hydrocefalie, 'cobblestone' (voorheen type II) lissencefalie, dysplasie van het cerebellum, agenesie van de vermis, Dandy-Walker cyste en een in aanleg gestoorde oogontwikkeling.

In Hoofdstuk IV worden bijzondere vormen van CMD beschreven welke niet in het nieuwe classificatiesysteem kunnen worden ondergebracht. In Hoofdstuk

IV paragraaf 1.1 wordt een familie beschreven met een (waarschijnlijk?) autosomaal dominante vorm van congenitale spierdystrofie. Dit verslag betreft een vader en dochter met CMD zonder aandoening van het centrale zenuwstelsel. De histologische bevindingen, vooral enige mitochondriële afwijkingen in het spierbiopt van de vader zijn opmerkelijk.

In Hoofdstuk IV paragraaf 1.2 worden twee zusters beschreven met ernstige degeneratieve kenmerken van het centrale zenuwstelsel, in samenhang met een neuromusculaire aandoening met histologische kenmerken van CMD. De pathologie van de hersenen verschilde wezenlijk met die van de reeds eerder in verband met CMD beschreven patiënten, namelijk een subtotaal verlies van neuronen in de cerebrale en cerebellaire schors alsook in het ventrale deel van de pons, en een secundaire teloorgang van myeline in de subcorticale witte stof van cerebrum en cerebellum. Deze afwijkingen worden beschouwd als een zeer vroegtijdig optredende degeneratie van meerdere systemen binnen het centrale zenuwstelsel. De nervus suralis vertoonde afwijkingen in de zin van een tekort aan vezels met een grote diameter, hetgeen niet wordt beschreven bij F-CMD. Binnen het spectrum van F-CMD zouden deze twee patiënten mogelijk aan een zeer ernstige vorm kunnen lijden.

In Hoofdstuk IV paragraaf 1.3 wordt een Nederlands meisje beschreven met congenitale spierdystrofie gecombineerd met hypoplasie van de laterale buikwand musculatuur en hypoplasie van de uitwendige genitaliën.

In Hoofdstuk IV paragraaf 2.1 wordt een Nederlandse jongen beschreven met een geïsoleerde dystrofie van de diafragma musculatuur, die een lichte postnatale asphyxie vertoonde en later perioden van insufficiënte ademhaling bij het doormaken van virale infecties.

In Hoofdstuk IV paragraaf 3.1 worden drie familieleden beschreven met een op volwassen leeftijd beginnende en voornamelijk distale spierzwakte. Bij de vrouwelijke patiënt was sprake van epilepsie en een spastisch-atactische gang, terwijl de twee andere familieleden geen waarneembare aandoening van het zenuwstelsel hadden. Bij alle drie was sprake van spierdystrofie en leukoencephalopathie.

In Hoofdstuk V, Algemene Discussie, hebben we onze resultaten opnieuw afgezet tegen de achtergrond van de meest recente classificatie van CMD van 1995.

Dankwoord

Dit onderzoek werd verricht vanuit het Interdisciplinair Kinderneurologisch Centrum (hoofd: Prof.Dr. F.J.M. Gabreëls) van het Academisch Ziekenhuis Nijmegen St Radboud. De studie maakte deel uit van het research-programma "Aandoeningen van het Neuromusculaire Systeem" van de Universiteit van Nijmegen.

Hierbij wil ik gaarne allen hartelijk danken die aan de totstandkoming van dit proefschrift hebben bijgedragen. Enkele personen en afdelingen wil ik met name noemen:

- Prof.Dr. F.J.M. Gabreëls, promotor, beste Fons, je scherpzinnigheid, inzet, creativiteit en vooral ook je gastvrijheid gedurende de afgelopen tien jaren heb ik zeer gewaardeerd.
- Dr. W.O. Renier, co-promotor, beste Willy, hartelijk dank voor je voortdurende stimulans, je scherpe analytische en redactionele kritieken en -last but not least- je onvoorwaardelijke steun.
- Dr. H.J. ter Laak, co-promotor, beste Henk, hartelijk dank voor je exacte bewerking en enthousiaste bespreking van alle morfologische gegevens en met name de methodologische benadering ervan.
- Prof.Dr. P.G. Barth, het Kinder AMC Amsterdam, beste Peter, en Dr. K. Renkawek, beste Kristina, een bijzonder woord van dank voor jullie belangrijke en voor mij leerzame bijdrage aan de studie van de cerebrale pathologie.

- Staf en medewerkers van het Interdisciplinair Kinderneurologisch Centrum (hoofd: Prof.Dr. F.J.M. Gabreëls).
- Staf en medewerkers van het Instituut voor Kindergeneeskunde (hoofd: Prof.Dr. R.C.A. Sengers).
- Staf en medewerkers van het Research Laboratorium voor Morfologische Neurologie (hoofd: Mevr.Dr. K. Renkawek) van het Instituut voor Neurologie.
- Staf en analisten van het Laboratorium voor Kindergeneeskunde en Neurologie (hoofd: Prof.Dr. J.M.F. Trijbels).
- Staf, laboranten en andere medewerkers van de afdeling Klinische Neurofysiologie (hoofd: Prof.Dr. S.L.H. Notermans).
- De staf van de sectie Klinische Genetica (hoofd: Drs. B.C.J. Hamel), in het bijzonder Dr.H.G. Brunner, van de afdeling Anthropogenetica.

- De afdeling Neuropathologie (hoofd: Drs. P. Wesseling) van het Instituut voor Pathologie.
- De afdeling Neuroradiologie (hoofd: Prof.Dr. H.O.M. Thijssen) van het Instituut voor Radiodiagnostiek.
- Staf en medewerkers van het Instituut voor Neurochirurgie (hoofd: Prof.Dr. J.J. van Overbeeke).
- Mevr.Dr. C.M. Mooy, Laboratorium voor Klinische Pathologie van het Academisch Ziekenhuis Rotterdam.
- De heren N.G.J.M. Dijkstra en J.H.G. Wijnen van de voormalige Foto- Film- en Teken afdeling van het Instituut voor Neurologie.
- Mevr. J. Abma-Hill voor correcties van de Engelse taal.

In het bijzonder wil ik de heer A.C. Romsom persoonlijk bedanken voor zijn betrokkenheid bij deze studie en voor zijn vele en deskundige adviezen alsook zijn grote accuratesse ten aanzien van de inhoud en de technische uitvoering van dit proefschrift.

Curriculum vitae

De auteur van dit proefschrift werd geboren op 11 november 1954 te Tilburg. Na het behalen van het diploma Gymnasium beta aan het Mill Hill College te Goirle, studeerde hij – door de speling van het lot – van 1974 tot 1975 Geneeskunde aan de Katholieke Universiteit van Leuven. Daarna begon hij met de studie Geneeskunde aan de Erasmus Universiteit te Rotterdam, alwaar hij in juni 1982 het artsexamen behaalde.

Tijdens zijn studie was hij werkzaam op de afdeling Neuro-anatomie en Fysiologie van de Erasmus Universiteit te Rotterdam (Prof.Dr. R.N. Lemon, thans Oxford University, en Prof.Dr. H.G.J.M. Kuypers †).

Van juli 1982 tot juli 1986 volgde hij de opleiding tot neuroloog aan het Instituut voor Neurologie van het Academisch Ziekenhuis Nijmegen (opleider: Prof.Dr. B.P.M. Schulte †). In het tweede deel van 1986 was hij werkzaam als neuroloog op de afdeling Neuro-Intensieve Zorg in voornoemde kliniek.

De aantekening Klinische Neurofysiologie werd in 1987 behaald (opleider: Prof.Dr. S.L.H. Notermans).

Van 1 januari 1988 tot 1 januari 1994 was hij werkzaam als neuroloog in het Streekziekenhuis Midden-Twente te Hengelo. Sedert 1 januari 1994 is hij werkzaam als neuroloog en B-opleider in Ziekenhuis Rijnstate te Arnhem.

STELLINGEN

behorende bij het proefschrift

CONGENITAL MUSCULAR DYSTROPHY

Clinical and morphological studies

in het openbaar te verdedigen
op 19 januari 1996
des namiddags te 1.30 uur

door

Q.H. LEIJTEN

1 De classificatie van congenitale spierdystrofie berust primair op de klinische symptomatologie De definitieve indeling zal in de toekomst door het moleculair genetisch onderzoek worden bepaald
– dit proefschrift

2 Bij kinderen met een ernstige cerebrale aandoening en structurele oogafwijkingen verdient het aanbeveling CK te bepalen ter opsporing van een mogelijke spierdystrofie
– dit proefschrift

3 Patienten met congenitale spierdystrofie en structurele afwijkingen van de ogen en van de hersenen kunnen worden onderscheiden in een groep met ernstige klinische presentatie en progressief beloop (Walker-Warburg syndroom) en in een groep met mildere klinische presentatie en minder progressief beloop (Finse vorm van 'muscle-eye-brain disease') Het is niet bekend of deze groepen genetisch heterogeen zijn of niet
– dit proefschrift

4 Een vorm van epilepsie of een epileptische predispositie bij EEG-registratie kan deel uitmaken van de merosine-negatieve congenitale spierdystrofie met witte stof afwijkingen in het centrale zenuwstelsel
– dit proefschrift

5 'Times are changing' "Een geneesheer vraagt in *Zeitschr f Krankenanstalten,* of hij schadevergoeding kan eischen van een patiente, die bij het ondergaan van een tandheelkundige bewerking een krampaanval had gekregen en hem een instrument in het oog had geslagen, zoodat hij enige dagen niet had kunnen werken "
– Pinkhof H, Ned Tijdschr Geneeskd 1919, Eerste helft, No 22, bl 1973

6 "Retinal – or ophthalmic – artery vasospasm should be considered as the cause of amaurosis fugax when thromboembolic disease and carotid-artery hypoperfusion have been carefully excluded as causes In such cases, a calcium-channel blocker may be effective treatment "
– Winterkorn JMS, *et al* N Engl J Med 1993,329 396-398

7. De verklaring van het ministerie van WVC dat dihydroergotamine en ergotamine 'goed werkende en veilige alternatieven voor sumatriptan' zijn, is onjuist en niet gebaseerd op wetenschappelijk aangetoonde feiten.
   – Ferrari MD, *et al.* Ned Tijdschr Geneeskd 1993;137:846-855

8. Het is onjuist dat het 'numerus fixus' systeem voor geneeskunde zich in Nederland al meer dan 20 jaar op cijfers fixeert, en volledig voorbijgaat aan sociale – en andere – vaardigheden.

9. Indien het vermoeden bestaat op een spieraandoening, verdient het aanbeveling een spierbiopsie te laten verrichten in een centrum dat gespecialiseerd is in neuromusculaire aandoeningen.

10. Een gevolg van de invoering van de Algemene Maatregel van Bestuur van 1 februari 1993 met betrekking tot het aantal werkuren voor arts-assistenten is de discontinuïteit, waardoor de kwaliteit van de zorg en van de opleiding wordt bedreigd. Door de opgelegde beperking in aanwezigheid ervaart de assistent in opleiding niet voldoende de verantwoordelijkheid voor de aan hem toevertrouwde patiënt.
    – Roos, RAC. In "Statistisch dynamisch, neuron en neuroloog", inaugurele rede, 3 maart 1995, Rijks Universiteit Leiden

11. "There are lies, damned lies and statistics, in this order."
    – Benjamin Disraeli (1804-1881)

12. De jeugd eindigt daar, waar men relaties gaat maken in plaats van vrienden.